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The Environmental Impact of

Crassula helmsii

by

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for the Degree of Doctor of Philosophy

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Abstract

The aquatic macrophyte *Crassula helmsii* is a non-native species and classed as 'invasive' in the UK. This study was carried out in an attempt to provide empirical evidence as to whether the establishment and growth of *Crassula helmsii* has a measurable impact on the ecology of invaded sites. Dispersal and distribution patterns across England were explored using databases and GIS interpretation. Ecological impact was measured by comparing invaded sites with uninvaded control sites on a range of habitats and waterbody types across Kent and East Sussex.

The analysis of spread patterns provided evidence that the plant is likely to have been dispersed due to the horticultural trade, as well as natural vectors into neighbouring habitats. Macrophyte analysis showed that species losses did not occur when *C. helmsii* was present. Changes to species composition did occur, with rarer plant species being associated with the presence of *C. helmsii*. Freshwater macroinvertebrates showed no change in either species number or species rarity. The seed banks of invaded and control sites showed no difference, but active management was shown to reduce the total number of seeds in the soil significantly. The water chemistry of invaded and uninvaded sites showed a relationship between the presence of *C. helmsii* and reduced total organic nitrogen.

The results of this study show that the expected species loss associated with non-native species may not be occurring with *C. helmsii*. Active management may be impacting the ability of native species to recolonise. Further work on other habitats and waterbodies across a wider geographic range are required to explore whether this is a localised effect.

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Finally I thank my research assistant and partner Laura Edey, who tolerated a range of weather conditions and expeditions across reserves with only minimal moaning, allowed the fridge, freezer and house in general to be commandeered by samples and tolerated continual rants about non-native species getting unfair treatment.

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Chapter 1 – An Introduction to *Crassula helmsii*

Origins

Crassula helmsii is a plant native to Australia and New Zealand (Child and Spencer-Jones, 1995; Preston and Croft, 1997). In Australia it is present across the country apart from the far north (Laundon, 1961; Dawson and Warman, 1987). Initial confusion was caused with identification and nomenclature of the plant, due to several names being used for the same species. The two earliest used were *Tillaea helmsii* and *Tillaea recurva* (Laundon, 1961). Further inspection identified them as the same plant, but different names have been used interchangeably.

In the early 20th century, the species was sold under the name *T. recurva*, which may have led to its classification under this name by naturalists. This was also the name used for the plant in Australia and therefore may have led to the adoption of that name in the UK (Lousley, 1957). A number of *Crassulaceae* species were present in the native range, and therefore, designation of the species into its own definitive nomenclature was reported as a difficult operation (Laundon, 1961).

The species can still be found under a range of synonyms, with the most often noted being *Tillaea helmsii* (Weber, 2003) and *Tillaea recurva* (Waal *et al.*, 1994). It seems likely that the source of these two separate names is due to different names from its Australasian source, with *T. recurva* originating from Australia and *T. helmsii* from New Zealand. The name *Crassula helmsii* (Kirk) Cockayne is currently the most common (Dawson and Warman 1987; Preston and Croft, 1997; Dawson, 1994; Child and Spencer-Jones, 1995; Leach and Dawson, Watson; 1999, 2000; Weber, 2003; Langdon *et al.*, 2004). *C. helmsii* is also known by its common names of Australian Stonecrop, Australian Swamp-Stonecrop, New Zealand Pygmyweed, Pygmyweed or simply as “Crassula”.

Further confusion exists with the name *Crassula helmsii* within the UK. *Crassula aquatica* (Northen Pygmyweed) is a rare Red Data Book species that was thought to exist in only a single location in Britain (Dawson and Warman 1987; Preston and Croft, 1997; Leach and Dawson, 1999). A more recent inspection of the records shows the species to present in four locations in Britain (NBN Gateway data access, 2012). This species may be confused with *C. helmsii,* due to it inhabiting the same aquatic habitats. *Crassula tillaea* (mossy stonecrop) may be confused

with *C. helmsii* due to the confusion of sharing the same genus. Identification between the two, however, is not difficult; being that *C. tillaea* is a succulent not present in aquatic systems.

C. helmsii has been available as an oxygenating pond plant in the horticultural ornamental trade since 1927 (Preston and Croft, 1997). It was noted as being sold as a 'Submerged Oxygenating Aquatic', in the 1960's species catalogue of 'Perry's Hardy Plant Farm, Enfield Middlesex, under the name *Tillaea recurva* (Laundon, 1961). It is thought to have been available from this supplier under this name since 1927 (Laundon, 1961). No other outlets were found to have supplied the plant under any of its pseudonyms, so it is thought that the sole origination of the plant into the UK is from this single horticultural supplier.

Introduction to the UK

The first presence of *C. helmsii* in the UK dates back to its introduction at Perry's Nursery before the First World War. In 1982, investigations were carried out to ascertain the location of the nursery (Swale and Belcher, 1982). Correspondence between the nursery and the authors of the paper were informed of its continued sale under the name *T.recurva*. It was also discovered, after correspondence with Mr R.H.Perry (a previous owner of Perry's Hardy Plant Nursery), that the plant was thought to have been brought back to Middlesex by Mr R.H.Perry's father before the 1914-18 conflict. Due to the neglect of the nursery tanks during the First World War (possibly due to the enlistment of Mr Perry senior into the armed forces), *C. helmsii* became abundant, 'choking out' (Swale and Belcher, 1982) the *Nymphaea* species that were likely to have been the ornamentals under cultivation. The subsequent continuation of the nursery provides evidence that *C. helmsii* fragments, growing amongst the other cultivated specimens, as well as the plant, may have been supplied to customers after the war, and may be responsible for its spread throughout the country through the horticultural trade.

C. helmsii was first found naturalised in Greensted, Essex, in 1956 (Laundon, 1961), followed by a report at the University of Southampton in an artificial pond in 1957 (Lousley, 1957). The source of the plant at Greensted was thought to be due artificial planting, but with suggestions that it could also have been through naturalisation from surrounding vegetation (Lousley, 1957; Laundon, 1961). The source of the plant at Southampton was reported as Perry's Nursery (Laundon, 1961).This was followed by reports across the country as identification amongst naturalists of a new species began (Bowman, 1977; Hall, 1978; Vaughan, 1978; Clement, 1979; Cockerill, 1979; Richards, 1979; Byfield, 1984).

Morphology

Crassula helmsii is a succulent aquatic perennial of the *Crassulaceae* family (Preston and Croft, 1997; Weber, 2003). In its native range, it is confined to areas with precipitation levels of 10 – 55cm in summer and 20 – 30cm in winter (Leach and Dawson, 2000). It is able to tolerate desiccation, so can occupy temporary ponds, which is most likely due to the xerophytic nature of its family grouping (Dawson and Warman, 1987). Its mean daily temperature requirements are a winter range of 0°C - 15°C (Fig. 1.1), with a summer range of 20°C - 25°C (Leach and Dawson, 1999), making it well suited to the climate of Britain and more specifically the south east. It can also tolerate prolonged spells of freezing (Kirby, 1965).

Figure 1.1. *C. helmsii* appears to remain viable in low temperatures, though the success of asexual reproduction from nodal fragments after freezing is unknown.

C. helmsii is thought to be an annual in its native range (Toelken, 1981). It shows signs of dieback in winter (Fig. 1.2), and so is classed as a perennial herb in Britain (Laundon, 1961; Dawson and Warman, 1987; Child and Spencer-Jones, 1995; Leach and Dawson, 1999). It

remains green throughout the year in the terrestrial, submerged aquatic and emergent aquatic forms (Hussner, 2009).

Figure 1.2. Photographs of the same pond in Summer (left) and Winter (right) within the Blean Woodland complex in Kent.

The leaves are opposite on the stem, sessile and connate at the base with an entire margin. This differs from the *Callitriche spp*. (Water Starworts), which have a notched leaf tip, which aids with identification in the field (Preston and Croft, 1997). This leaf notch is a useful tool in differentiating them with *C. helmsii*, which can initially look similar in growth forms. Another beneficial identification aid is a collar around the join of the leaf pair onto the stem (Brunet, 2002).

Rooting can occur from all nodes that are submerged or in contact with moisture, with a greater prevalence of roots being shown deeper in the water column (Leach and Dawson; 2000, Brunet, 2002). It is able to grow both roots and shoots from the same node (Fig. 1.3), giving it the advantage of a branched network of stems that can be supplied by a complex root system (Smith, unpublished work).

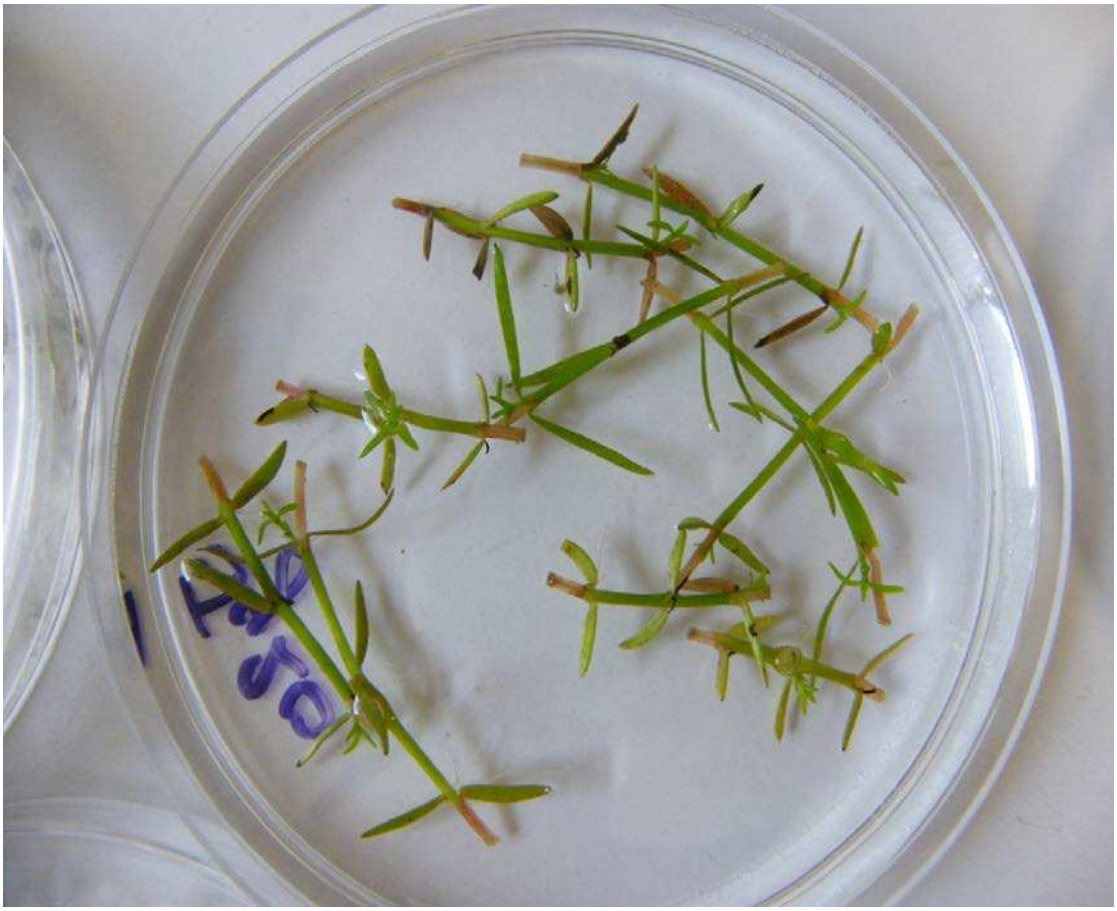

Figure 1.3. Re-growth of *C. helmsii* from 3-node section in vitro, showing new shoot growth.

Flowers

The flowers are stalked and borne individually (Leach and Dawson, 1999) in the axils of the leaves on pedicels of 2-8mm (Weber, 2003). They are generally white, though with examples of red/pink variations occurring (Dawson and Warman, 1987). Though generally consisting of 4 petals (Vaughan, 1978) with a diameter of 3-4mm, a minority have been shown to bear a variation on this of 3, 5 or 6 petals (Swale and Belcher, 1982). Floral variation has been shown to be a common trait (Swale and Belcher, 1982). Other variations include a pink corolla and bending of the petals in a reflexed or recurved manner over the carpels. Swale and Belcher (1982) note this as a distinguishing feature and an aid to identification. Due to the narrow window of flowering times from August-September (Laundon, 1961; Dawson, 1994), it is something that may be useful as an additional rather than sole identifying method.

The flowers are also noted as releasing a fragrance, which is thought to aid pollinator attraction in its native range (Dawson and Warman, 1987). No pollinators have been identified for the species in the UK.

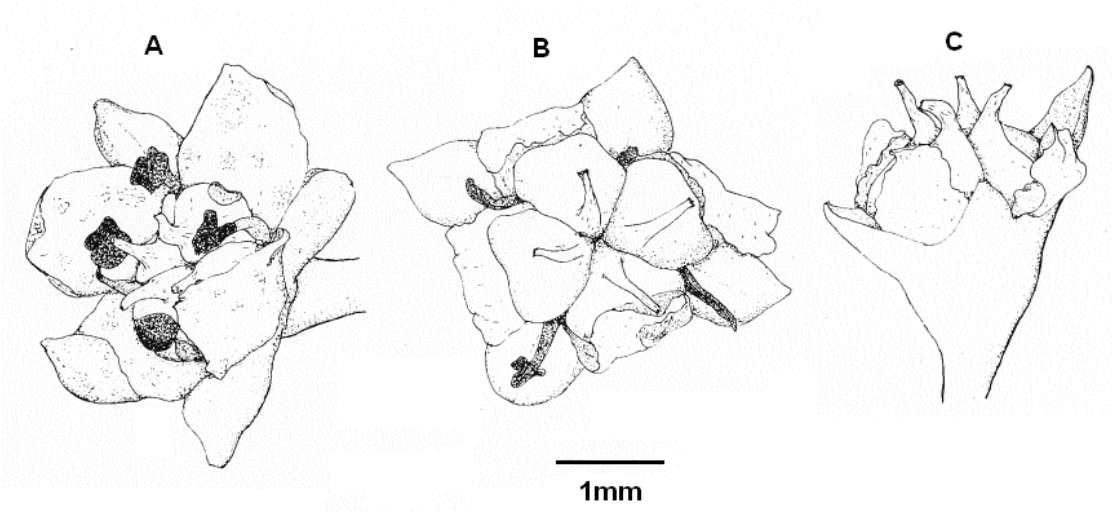

Figure 1.4. *C. helmsii* growth forms and morphology sketches (Taken from Richards, 1979). A= Flower Head, B + C = Young fruit.

Seeds

Seeds have been shown to be produced, with carpels nearing maturity containing approximately 25 seed embryos (Dawson and Warman, 1987). Weber (2003) provides a different opinion, describing each fruit as containing 2-5 elliptical seeds within each. Whether this is a difference between its reproductive ability in Australia (Dawson and Warman, 1987) compared to the situation in the UK (Weber, 2003) is not clear from the literature.

As initial germination was not achieved in early research, it was thought that although seeds were produced, they remained sterile. Viability tests were carried out in the mid 1980's on seeds (Dawson and Warman, 1987). Seeds were found to float initially, but had a tendency to sink over time, which provided evidence of a possible dispersal mechanism. Sampling of the riparian soils allowed for isolation of the seeds and the discovery of split seed cases, but no evidence of germination was discovered.

More recent work has found that germination of *C. helmsii* seeds (Fig. 1.5) may be achieved (Denys *et al*., 2014) using inflorescences as a method of seed inoculation. It was found that germination success was greater by 18% when the inflorescences were placed near or at the

surface, compared to being buried by 30-40 mm (Denys *et al.*, 2014). This study concluded that dispersal by seeds should be considered as a credible method of dispersal, but with asexual fragmentation remaining the main method of spread. The use of inflorescences rather than the isolated seeds may also have included nodal fragments attached to the inflorescence. This would be difficult to distinguish from direct seed germination, and may have led to the success of the trials in this case. Further studies on seed germination abilities are needed to support these findings.

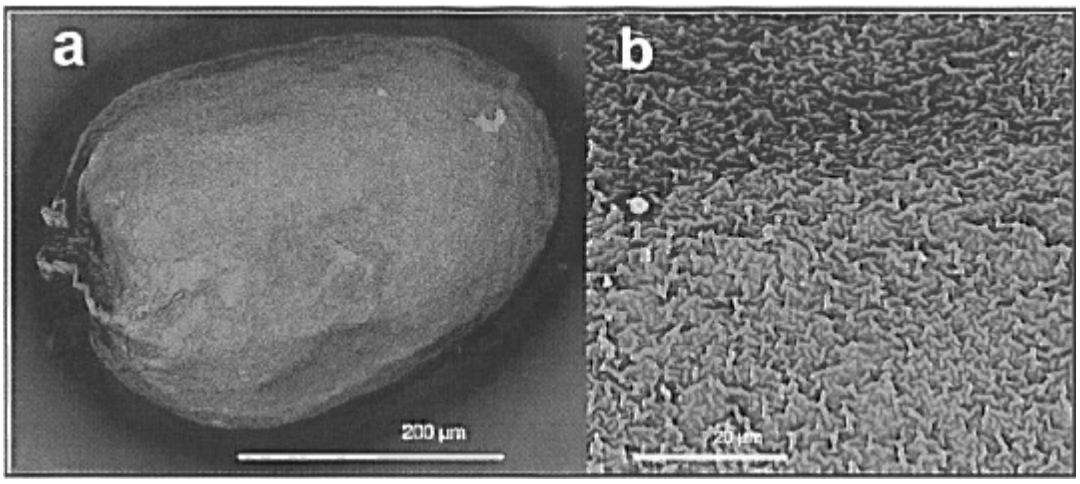

Figure 1.5. A seed of *C. helmsii* and surface texture of the seed. (Taken from Denys *et al.*, 2014.)

Growth Forms

C. helmsii can exhibit a range of growth forms due to its ability to colonise three separate habitat types (Fig. 1.6). They are roughly categorised as terrestrial, emergent on the water's surface and a fully submerged form (Dawson and Warman, 1987; Dawson, 1994).

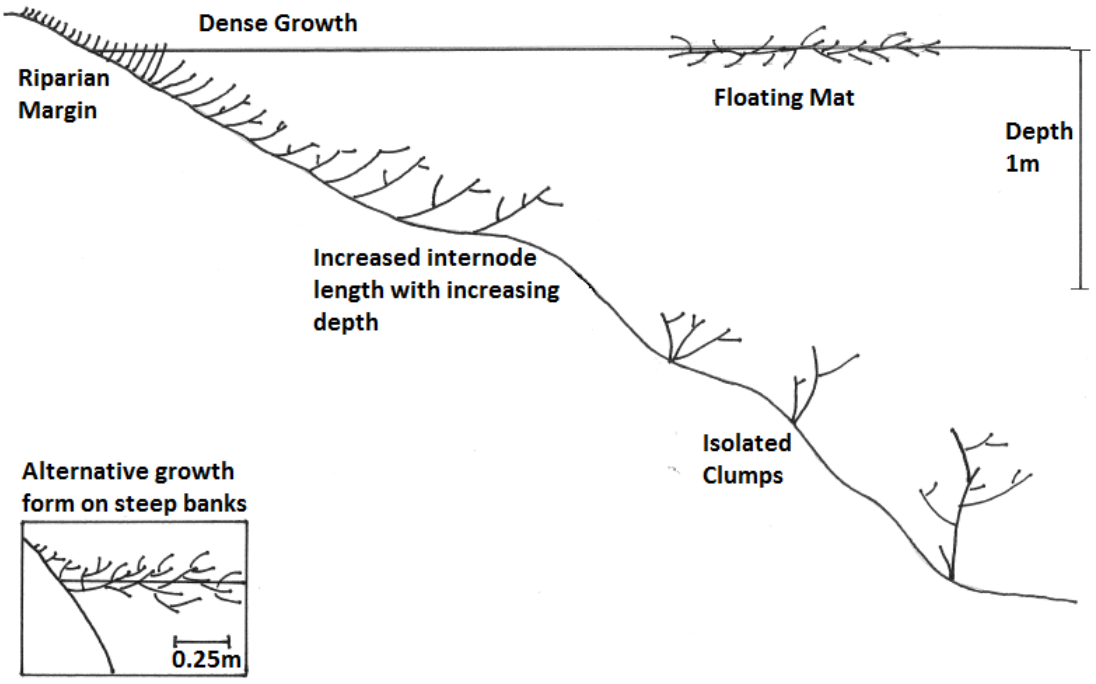

Figure 1.6. Different growth forms of *C. helmsii* in naturalised settings (Taken from Dawson and Warman, 1987).

The tissue growth of the plant is specialised, dependant on which of the growth forms it exhibits (Dawson and Warman, 1987). These are non-exclusive, with one stand of the plant often exhibiting all of the forms, leading to difficulties in identification. The terrestrial form generally shows features similar to that of true xerophytes within the *Crassulaceae* family (Taiz and Zeiger, 2006). This growth form is generally shorter, with thick 'succulent' leaves of 4 -15 mm (Child and Spencer-Jones, 1995) that are short in comparison to the other growth forms (Dawson and Warman, 1987). The spread of the creeping stems, which exhibit a lateral growth form, reaches a maximum spread of 0.3-0.4 m (Dawson, 1994). The height of the plants at this stage generally does not exceed 5 cm (Pysek, 1995). However, due to the self-supporting and intertwining nature of the plant, these stands will increase in height, often reaching in excess of 10 cm. The intermodal length is the shortest of all the growth forms, giving it a dense 'shrubby' appearance not dissimilar to some of the Mediterranean *Sedum sp*. or woodland mosses such as *Polytrichum commune* (Common Hairmoss).

The emergent form of growth is similar to that of the terrestrial form when viewed above the water level. Below the water (0.5-1 m) the plant changes significantly on the same stem section (Fig. 1.7). Multiple branch sections are present, with the plant often forming a dense sward. This may remain close to the bank, or extend further out creating a dense floating

mass. This may be anchored to the bank, but is liable to breaking and dispersing through wind action, with possible implications towards spread dynamics (Dawson and Warman, 1987). The transformation from one growth method to another on the same plant section, shown by tank culture trials, has been shown to be a rapid process (Dawson and Warman, 1987).

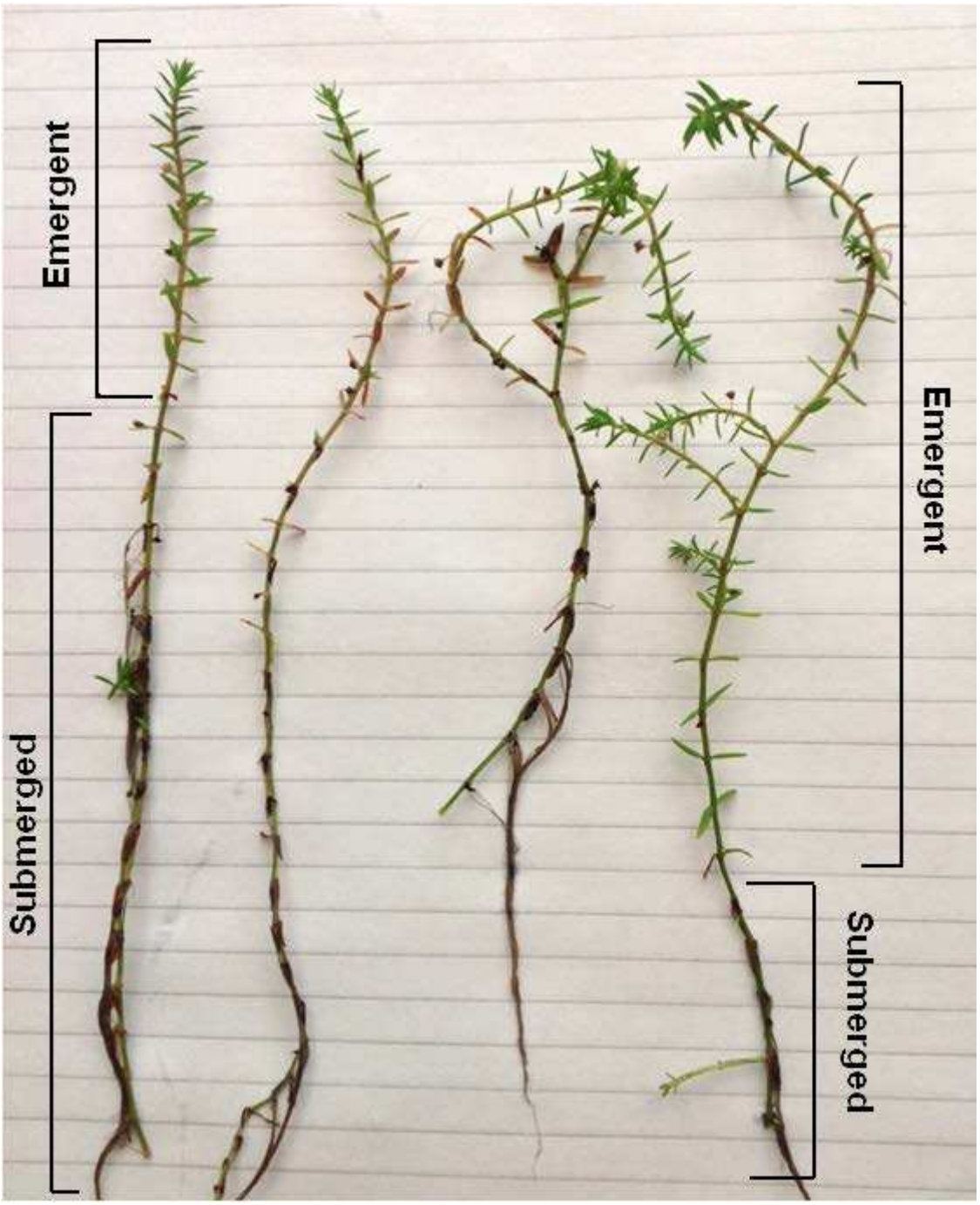

Figure 1.7. The variation in morphology of *C. helmsii* on the same stem.

The change from emergent growth to submerged growth form is seen with clear distinction. Shorter node spacing and thicker leaves are typical of the emergent form, whilst the submerged form shows a dominance of root growth and less foliar presence.

The fully submerged growth form extends to a maximum of 3 m in depth. The leaves become narrowed to 0.3-0.4 mm (Dawson and Warman, 1987). This may be explained by the fact that in its native range, it can be found in riparian habitats under flowing water conditions (Dawson, 1989). It may also provide a form of energy saving through decreased tissue growth in relation to a reduction in surface are, which relates to a reduction in light transmittance with increasing depth.

The fully submerged form shows an extension in internode length to a maximum of 20 – 25mm (Dawson and Warman, 1987). This becomes shorter (15 – 20 mm) towards the apical end of the plant. This is an increase of 3 times that shown by the emergent growth form (Dawson, 1994). The stem length is a maximum of 1.2 m (Dawson, 1994) to 1.3 m (Dawson and Warman, 1987), with branching patterns following a similar trend to that of the internode length of a greater network of growth towards the surface.

Habitats

The range of habitats that *C. helmsii* can grow on is limited to aquatic/wet freshwater or brackish systems, with no recorded growth in saline conditions (Dawson and Warman, 1987; Dawson, 1989; Brunet, 2002). It is thought that high saline content can limit its distribution (Brunet, 2002). This has been used as a control strategy (Charlton *et al.*, 2010; Dean *et al*., 2013). Within these habitats are a range of smaller sub-habitats where it can thrive. These include; wet mud, small ponds, shallow gravel sand or clay lakes, linear watercourses with no flow, bankside vegetation of flowing linear water courses, damp ground and marsh (Dawson, 1994; Child and Spencer-Jones, 1995,). The pond systems can be further subdivided into garden ponds, agricultural ponds and nature reserve ponds (Dawson and Warman, 1987). Though seemingly favouring alkaline conditions, *C. helmsii* has also been noted growing in acidic bog systems, both in the New Forest (Brunet, 2002) and in this study at Hothfield Heathlands, Kent.

In studies of its distribution and physiology in the UK, *C. helmsii* has been shown not to grow in flowing water. This is different to its native range, where it can be found in flowing waters and

their subsequent riparian habitats (Dawson, 1989; Leach and Dawson, 1999). In tank trials, flowing water speeds up to 0.3 ms^{-1} resulted in an increase in biomass of plant tissues (Dawson and Warman, 1987). Though the tank trials appear to differ to field studies, an interaction with the added nutrient within these tank experiments may explain the increased growth (Dawson and Warman, 1987). Why growth in flowing water in the UK has not been encountered to date is yet to be explained.

C. helmsii can reach densities of up to 1 kg dw. m^2, due to the multiple stem branching that occurs both in the aquatic and terrestrial form (Hussner, 2009). The emergent form of the plant reaches maximum gas exchange under low light and low temperature ranges (Hussner, 2009). This factor may be the reason for its success and rapid spread in Britain, which exhibits these climatic conditions regularly. The ability for *C. helmsii* to grow in low light levels, and its continuous photosynthesis under normally sub-optimal conditions, has led to it being described as growing in a 'diesel' type manner (Newman, 2013).

CAM Metabolism

C. helmsii is adapted to use crassulacean acid metabolism (CAM). CAM is a method utilised by both aquatic and xerophytic species. CAM is also used by the native *Crassula* species in Britain, *Crassula aquatica* (Northern Pygmyweed) (Newman and Raven, 1995, Preston and Croft, 1997). It is most often reported in the *Isoetes* genus (Keeley and Morton, 1982). It is thought to be present in approximately 6% of the tested aquatic macrophytes, which is thought comparable to the terrestrial biome (Klavsen and Maberly, 2010). CAM enables a plant to take up carbon dioxide during the night; therefore enabling stomata to remain closed during the day and prevent losses from evapotranspiration (Winter and Smith, 1996, Klavsen and Maberly, 2009). A typical example of water loss for CAM plants is 50 to 100g for every gram of CO_2, in comparison to C4 plants which lose 250 to 300g, and C3 plants, which lose 400 to 500g (Taiz and Zeiger, 2006).

The inorganic carbon that is taken up is stored as malate, as a temporary substrate within the vacuole (Klavsen and Maberly, 2009). During the following day, the malate is decarboxylated, with the released CO_2 entering the Calvin cycle via ribulose-1,5-bis-phosphate carboxylase-oxygenase. The diel-rhythm of acidification at night compared to the de-acidification during

the day can be used as a measure of the activity of CAM, being that the ratio of H^+ to malate is in the order of 1:2 (Keeley, 1996). This is found through the measure of titratable acidity, which is then compared to the measure of malate/malic acid (Newman and Raven, 1995).

For aquatic species, it would seem ecologically redundant to use CAM, being that there is no cost to the plant due to water being in excess. Therefore, additional theories have been suggested, such as aquatic environments being limited in CO_2, and so CAM metabolism may act as a 'carbon-conserving' mechanism (Maberly and Madsen, 2002; Klavsen and Maberly, 2009; Klavsen and Maberly, 2010). It may also act as nitrogen conserving mechanism. Increased levels of CO_2 at the enzyme Rubisco's active site makes growth more efficient (Sage and Kubien, 2003; Klavsen and Maberly, 2009).

The use of CAM is thought to enhance the uptake of dissolved inorganic carbon (Newman and Raven, 1995; Maberly and Madsen, 2002), in the form of bicarbonate (HCO_3^-), in addition to CO_2. The theory was examined under a range of conditions (Newman and Raven, 1995). The results showed an assimilation of CO_2 at night, with the subsequent storage in the form of malic acid. This was shown to be decarboxylated during the day, when assimilation of DIC was found to be present, but only through the classical C_3 pathway. Newman and Raven (1995) discussed the ideas that *C. helmsii* was not limited by the availability of DIC in host waterbodies, and thus was not correlated to this in its distribution, in the same manner as the *Isoetes* genus appears to be.

CAM experiments with *C. helmsii* were carried out by Klavsen and Maberly (2009), who noted the lack of in-field measurements from the early study by Newman and Raven (1995). Their study was of a mesotrophic, soft-water lake in the Lake District, England. Measurements were made of in-situ CAM activity and decarboxylation through sample collection of *C. helmsii* shoot tip material. This was also the method of measuring photosynthetic and respiration rates. As with Newman and Raven (1995) titratable acidity was used as the measure of CAM activity. It was found that CAM activity increased between April to July, in both their field and laboratory experiments (Klavsen and Maberly, 2009). There was also a significant correlation between light level at depth and the rate of decarboxylation. This showed a greater level of CAM activity in *C. helmsii* higher in the water column, as well as emergent forms of growth. Light intensity was thought to be the cause of this, and is supported by previous studies (Newman and Raven, 1995). This may indicate a preference towards growth at higher levels in the water column, and growth forms of both emergent and marginal forms. The contribution by CAM to total daily photosynthesis was skewed towards the July sampling regime, which would correlate

with the most active growing period of *C. helmsii*. Values of 22% and 18% in April were compared with values by CAM of 42% and 31% in July. It was thought that this greater activity may have been due to the combined effects of increased light quantity and raised water and atmospheric temperatures, both of which are known to be regulating parameters in terrestrial CAM plants (Taybi *et al.*, 2002).

CAM metabolism was noted in the copper accumulation study of *C. helmsii*, as it is thought that copper may be correlated towards the level of malate within plant tissues (Küpper *et al.*, 2009). It was noted that CAM was more active at depth (Küpper *et al.*, 2009), which contradicts the study by Klavsen and Maberly (2009).

Klavsen and Maberly (2010) investigated a range of light and CO_2 treatments on the effects of CAM in *C. helmsii*. The results found that CAM appeared to be an effective mechanism for carbon assimilation at high light levels, but was not present at low light. It was also found that the contribution to the carbon budget was higher at low CO_2 levels in comparison to the higher CO_2 plants. The amount of CO_2 taken up via CAM at night was 0.74 to 2.94 times the amount of CO_2 lost in respiration (Klavsen and Maberly, 2009). This supports the idea that CAM acts to enhance inorganic carbon uptake when it is in limited supply. It also supports their previous findings, where CAM was most active in plants higher in the water column, and therefore receiving greater levels of sunlight. Laboratory experiments demonstrated a 13% greater contribution to the carbon budget by CAM in low CO_2 vs. high CO_2 grown plants (Klavsen and Maberly, 2010). It is stated that *C. helmsii* is not limited to high alkaline (high DIC) systems (Klavsen and Maberly, 2010), which is reflected by its ability to colonise habitats with a range of pH values. The ability for *C. helmsii* to maintain high levels of photosynthetic activity, even at levels of CO_2 down to 3mmol m^{-3} may act as a competitive tool against other macrophytes, allowing it to exploit environments where other species may find DIC to be a limiting factor.

Nutrient Requirements

The nutrient requirements of *C. helmsii* are relatively unknown. Research would appear to indicate that it is able to occupy a "broad range" of nutrient levels (Leach and Dawson, 2000). Dawson (1994) found that the water chemistry of 25 sites invaded by *C. helmsii* showed a general trend of greater biomass growth in sites with high nutrient and low alkaline waters. This was supported by Brunet (2002) who examined ponds within the New Forest, Hampshire, England. Brunet (2002) identified some possible trends towards potassium and phosphorus

acting as limiting factors towards growth of *C. helmsii*. He also noted the ability for *C. helmsii* to uptake large amounts of nutrients from a system, akin to the *Lemna spp*. (duckweeds) but makes no inference to where these assumptions are from, and so must be assumed anecdotal. Decreased phosphorus was thought to reduce monoculture establishment, but not initial colonisation. This study is limited in the variables that it examined due to time constraints explained by the author (Brunet, 2002). It is, however, the most in depth study of the water chemistry of both invaded and uninvaded control sites to date.

CAM metabolism has been shown to be independent of the levels of nitrogen and phosphorus (Klavsen and Maberly, 2009). This was thought to be due to the excess that was available to the plant within tissues, in excess of suggested values of 1.3%N and 0.13%P (Gerloff and Krombholz, 1966). The link between nutrient availability and growth has been studied, during laboratory trials in 2004-2006. These included four invasive aquatic macrophytes (Hussner, 2009):-

- *Crassula helmsii* (New Zealand Pygmyweed)
- *Hydrocotyle ranunculoides* (Floating Pennywort)
- *Ludwigia grandiflora* (Water Primrose)
- *Myriophyllum aquaticum* (Parrot's Feather)

The trials showed that a strong correlation existed between nutrient availability and total biomass (dry weight), irrespective of the substrate used for propagation. This relationship was also found when comparing nutrient availability with relative growth rate. Though it is speculative, Hussner (2009) makes a comparison to field systems showing the same trend, with an example given of Lake Fuehlingen, Germany. This lake was measured as containing up to 40mg N kg^{-1} soil, 2 mg P_2O_5 (extractable phosphate)per 100g and 27mg $P_2O_5P_{tot}$ (total phosphate) per 100g. It returned results of the highest biomass development out of the sites surveyed.

The plant has also been studied for its ability to accumulate copper. Copper is an essential micronutrient (Taiz and Zeiger, 2006), but elevated concentrations can lead to an inhibition of plant metabolic processes (Küpper and Kroneck, 2005). Copper was widely used as a pesticide in agriculture (Mitchell *et al*., 1990). Plant growth trials using a copper enriched water solution have shown that the plant is not capable of node regeneration when bathed in a solution in excess of 2 mgl^{-1} of copper (Smith, unpublished work). Reasons for this are stated as being through the insertion of the Cu^{2+} cation into the photosystem 2 reaction centre photosynthetic

pathway, as well as oxidative stress, and an overall inhibition of the photosynthetic light reactions (Küpper *et al.*, 2009).

Metal Accumulation

C. helmsii is a hyperaccumulator of copper, and is capable of accumulating it to 9,000 ppm in plant dry weight, compared to 0.6ppm from a control group of macrophyte species (Küpper *et al.*, 2009). Coping strategies are thought to exist for *C. helmsii* and high copper levels. Accumulation and compartmentalisation of the copper into certain tissues occurs, where it is held without causing harm towards the plant (Küpper *et al.*, 2009). This has led to thoughts of using the plant in an artificial planting – such as in phytoremediation of anthropogenically enriched copper sites. Phytomining may also be possible, where the copper may be extracted from the plant in an economically viable process due to the rising costs of the metal (Küpper *et al.*, 2009).

The process of accumulation is thought to be linked to CAM metabolism. Increased presence of malate during CAM (Newman and Raven, 1995; Taiz and Zeiger, 2006) acts as a copper ligand and thus would show an overall increase in accumulating ability. This may subsequently enable the plant to grow in copper rich areas and gain a competitive advantage over those excluded due to increased toxicity. The rapid bleaching of the tissues when copper is in excess and subsequent dehiscence of leaves from the main stem may serve as a sacrificing mechanism. This could prevent the toxic effects from affecting the rest of the plant whilst still allowing for some level of nutrient recovery through decomposition and reabsorption (Küpper *et al.*, 2009). This illustrates that, although *C. helmsii* is an adept hyper accumulator of copper, in extreme environments it too would succumb to decreased growth and death, which would agree with observations made (Smith, unpublished work). It has been shown through pH changes on a diurnal cycle, as well as carbon isotope discrimination ($\Delta\ ^{13}C$), that pH extremes make *C. helmsii* more susceptible to copper toxicity (Küpper *et al.*, 2009). This may demonstrate a generalist approach to its life strategy, in that it demonstrates both coping strategies for extreme environments, but when faced with a combination of both high copper and pH extremes, it is more susceptible. When compared to other aquatic macrophytes however, it would seem likely that it is still a superior competitor in copper rich environments.

Genetic Diversity

The only research to examine the genetic differences between UK populations of *C. helmsii* was carried out by Dawson (1994). Dawson studied UK plant samples and seven enzyme systems, collected across a wide geographic distribution. The results indicated only one strain of *C. helmsii* present within the UK, with little apparent polymorphism within the species.

Dawson also studied the genetic differences from the native range of *C. helmsii*, most probably during his study in Australia in the late 80's (though not stated as such) (Dawson, 1994). This study looked at 34 separate sites and 11 separate enzyme systems (Dawson, 1989). Differences were found only between the two enzyme systems malate dehydrogenase and malic enzyme. This geographically separated the plant specimens between the Northern Tablelands and New South Wales from other Australian plant specimens. The genetic study indicated the most likely source of the British strain to be from the River Murray area – though the paper indicates the lack of information that this discovery shows, being that this particular river system drains half of the Australian continent.

Growth Regulator Interactions

A study of the effects of growth regulators on the regeneration capacity of *Crassula helmsii* has been carried out in vitro (Kane *et al.*, 1993). This study used four specific parts of the plant; a single node, an internode section, a stem tip and a leaf blade. Their work demonstrated the well-developed capacity of *C. helmsii* to increase in biomass. From a single shoot tip after 28 days, the plant mass consisted of 62 shoots, 127 rooted notes and 254 lateral buds (Kane *et al.*, 1993).

The addition of synthetic cytokinin BA to the growing mass showed an increased growth rate of shoots and nodes, but an inhibition of shoot elongation, which produced more compact but greatly branched plant masses. Naturally occurring cytokinins did not produce an increase in growth of either shoot or node from the explant, and so was deemed a non-limiting factor to *C. helmsii* growth in natural systems. An explanation for this was given as the number of root producing nodes present, and therefore a high proportion of plant tissue able to biosynthesise endogenous cytokinin (Kane *et al.,* 1993).

Asexual Reproduction

Crassula helmsii has been shown to regenerate from single nodes, regardless of whether the leaves are attached or removed (Hussner, 2009). This was replicated *in vitro* in this study (Smith, unpublished work), where the removal of leaves caused a reduction in regrowth, but nodal regeneration remained significant (Fig. 1.8).

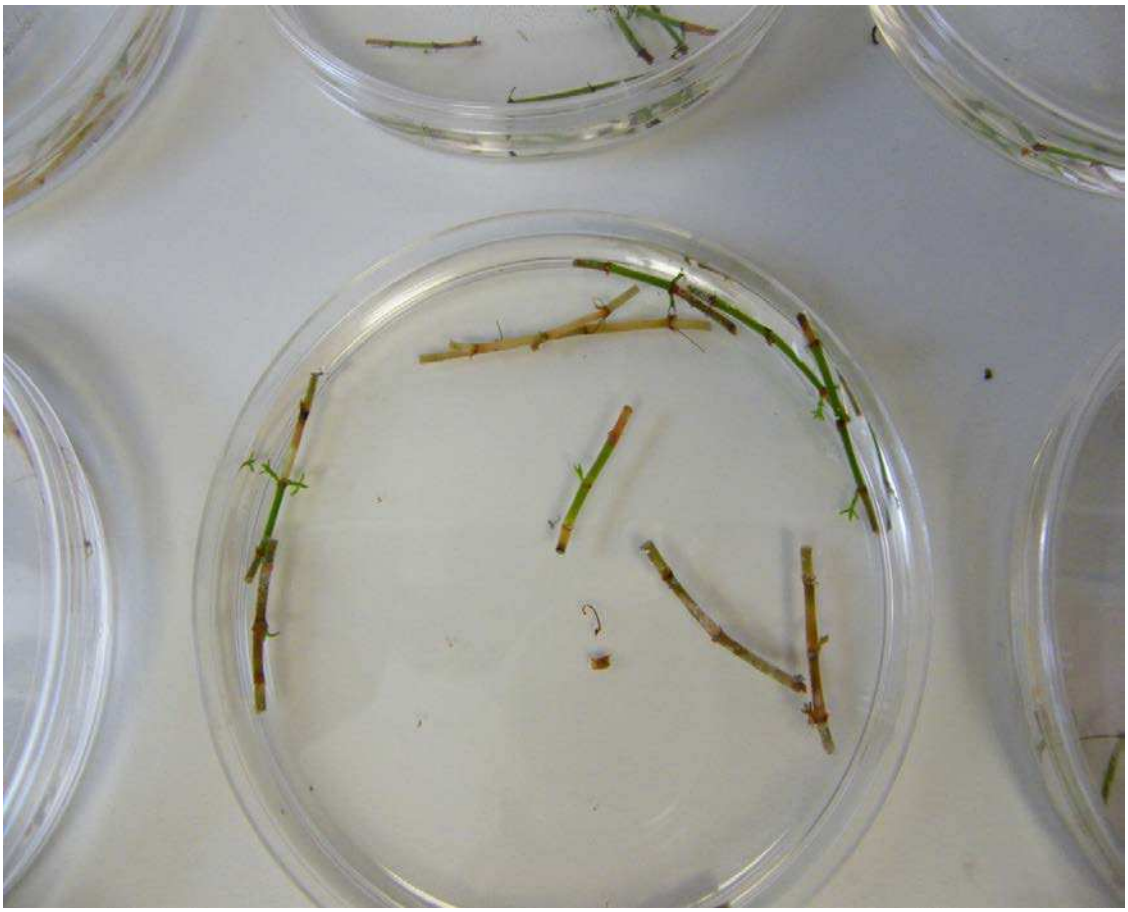

Figure 1.8. Stripped sections of *C. helmsii* showing foliar re-growth from the nodes.

Unlike *Ludwigia grandiflora* and *Myriophyllum aquaticum*, *C. helmsii* is not able to regenerate from individual leaves under normal conditions (Hussner, 2009). A comparative study of in-vitro growth found that individual leaf blades could be made to show adventitious shoot development (ASD) when supplied with the synthetic cytokinin BA and the naturally occurring cytokinins 2iP and Zeatin (Kane *et al.*, 1993). This method of propagation has not been detected in natural field systems however, which may be due to the synthesis location of cytokinin being in the root (Kane *et al.*, 1993; Taiz and Zeiger, 2006). This regeneration in vitro of internode tissue has also been tested with additive cytokinin of both synthetic and naturally derived forms (Kane *et al.*, 1993) with similar results to that of individual leaf blades. However,

the control experiments showed the ability for internode tissue not containing a node to still develop ASD, and so this may well be a further regeneration technique used in natural systems.

Dispersal Mechanisms

Dawson and Warman (1987) were the first to investigate the possible vectors of *C. helmsii* in their study sites in and around the New Forest, Hampshire, in 1986. These included Canada geese (wildfowl), fishing tackle and ponies (which roam wild on the interior of the New Forest, acting as a habitat management tool). It may also be inadvertently moved on the bottom of botanist's boots, during the study of the plant (Leach and Dawson, 1999).

The ponies were noted as 'grazing' the plant, but whilst they were proposed as a potential vector, no evidence of them acting in this way was provided (Dawson and Warman, 1987). This is supported by more recent work (Chatters, 2013), who thought that the ponies did not aid in dispersal directly. No mention of seed dispersal through ingestion is noted by Dawson and Warman (1987), but they do offer a morphological viewpoint on the lack of attachment structures on the seed casing. Movement via ponies, and other grazing livestock, is important in current nature reserve management. Grazing regimes, using a range of livestock that are well suited to thrive in wet conditions (such as Highland Cattle and Konik Ponies) are a feature of current conservation on wetlands. Their ability to control invasive scrub and create age structures in vegetation is seen as an important tool in tackling large sites, where the economics restrict human control. Though this may be the case, the prevention of spread of invasive species such as *C. helmsii* is often overlooked. Waterbodies are likely to act as 'bottlenecks' of animal movements through the necessity to drink, with the possibility of moving the propagules via adherence to hair and fur, between species as well as directly from the water. The consequence of this is the spread of the plant across entire reserves. Further spread may occur when livestock is moved between sites, which is a technique used by conservation bodies.

Mechanical control was the only method of control of *C. helmsii* in Germany, and is noted for its probable exacerbation of the spread of the plant due to the ease of propagation by individual nodes (Hussner, 2009).

Dispersal may also occur by 'accidental inclusion' from the aquatics trade, in both contaminated fish and plant stocks. Anecdotal evidence gives many examples of such vectoring of propagules, with pond owners often finding *C. helmsii* to be present in their gardens without intentionally releasing it. This was thought to be responsible for the growth of *C. helmsii* at Southampton University, one of the earliest records of the plant (Laundon, 1961). This is likely to be difficult to isolate from dispersal by factors such as wildfowl, with supplier inspection likely to be the only true way of identifying the vector. Its supply as an aquatic oxygenator (Laundon, 1961; Dawson and Warman, 1987) is likely to play a large part in its original cause of widespread dispersal, with one nursery thought to provide the source of the invasion in the UK (Swale and Belcher, 1982). The practice of stocking ornamental aquatics in large tanks makes large numbers of individual plants liable to inoculation with fragments. This could account for the movement into homes, but is unlikely to account for the widespread movement across nature reserves and the wider countryside (though possible 'public restocking' by well-meaning members of the public may lead to the same outcome).

The ability for *C. helmsii* fragments to remain viable after ingestion by wildfowl and be distributed by endozoochorus dispersal was found not to be a decisive factor, with only a single plant developing after incubation of wildfowl droppings (Denys *et al.*, 2014). A likely explanation for this is cross contamination from the exterior of the birds, which provides further evidence of ectozoochorus transmission.

Darwin (1878) studied species movement due to wildfowl, with an experiment using tanks of pond snails and the insertion of 'removed' water bird's legs, to test the level of adherence. The movement of *C. helmsii* via attachment to the appendages of wildfowl (ducks, geese and heron) visiting the water bodies is known to be a method of transmission (Denys *et al.*, 2014). Figuerola and Green (2002) noted the importance of water birds as a vector for species movement between waterbodies, and the lack of research that this line of enquiry has received. Both endozoochory (internal) and ectozoochory are discussed. Though the research for this method of transmission is noted as 'sparse', some anecdotal evidence of adherence to plumage is discussed (Figuerola and Green, 2002). Exposure to desiccation is noted as the most likely limiting factor, which would have minimal effects upon *C. helmsii,* with its ability to propagate from nodal fragments after extended periods of desiccation (Hussner, 2009). The ability for fragments to float is seen as an important factor to enhance the adherence ability to plumage (Figuerola and Green, 2002), which is noted as a trait of *C. helmsii* (Dawson and Warman, 1987.) Smaller fragments are also seen to be better at this than larger sections

(Figuerola and Green, 2002). Factors such as drinking, filter feeding and general trampling create an additive factor to the dispersal by water birds.

Environmental Impact

Environmental impact towards native species by invasives is the predominant concern of invasion (Dogra *et al*., 2010). Most evidence that suggest species losses and changes to species compositions is anecdotal with little data to support it (Byfield, 1984, Walters, 1996, Watson, 1999). It has been shown to impact native aquatic macrophytes in Germany, in both lakes and ponds, across its range (Hussner, 2009). The most in-depth study into the effects towards native species in the UK was carried out in relation to newt conservation (Langdon *et al.*, 2004). Effects were also noted by Langdon *et al.* (2004). In this study, *Lissotriton vulgaris* (Smooth Newt) showed a significant difference in developmental stage at hatching when offered either the preferred *Nasturtium officinale* (Watercress) or *C. helmsii* as an egg laying substrate. In the same trials, *Triturus cristatus* (Great Crested Newt) did not show this difference between plant substrates. This shows a relationship between substrate and development of the egg which is species specific (Langdon *et al.*, 2004). Reduced breeding success was noted in a pond invaded by *C. helmsii* in another study, thought to be due to the reduction in available submerged leaf material used for egg wrapping (Watson, 1999).

Langdon (2004) also examined the effects of *C. helmsii* towards native plant species. A significant effect was found with the inhibition of seed germination when *C. helmsii* was present within tank trials. In comparison to controls, germination was significantly reduced in:-

- *Epilobium hirsutum* by 83%
- *Lythrum salicaria* by 69%
- *Mentha aquatica* by 56%
- *Ranunculus sceleratus* by 51%
- *Veronica beccabunga* by 48%
- *Myosotis scorpioides* by 43%.

This could have indirect impacts on further species groups such as macroinvertebrates, but no studies have as yet investigated this.

Field systems (ponds in North West England) showed no evidence of species loss through suppression of germination. There was also no significant difference in the length of time that

C. helmsii had been present and the species diversity of the pond. Seed bank density showed no significant difference between *C. helmsii* invaded and control sites. The average numbers of seed species on invaded sites was 25.8 compared with an average of 24.8 seed species on control sites (Langdon *et al.*, 2004). This therefore demonstrated a different assessment of the impacts of *C. helmsii* between laboratory and field systems.

In a survey of 116 ponds in the New Forest (20% of the total number of ponds in the New Forest) a range of 123 macrophytes, including 18 rarities, were found (Ewald, 2013). All ponds showed biodiversity scores above the national average (Ewald, 2013). The results were similar for freshwater macroinvertebrates, with a range of 90 being found, including rarities. The majority of biodiversity scores for macroinvertebrates were above the national average (Ewald, 2013). The ponds surveyed consisted of 8 control ponds, with the remaining 108 showing presence of *C. helmsii*.

A further study of the effects of invasion was carried out at Broad Ees Dole nature reserve in Mersey Valley, UK (James, 1995). The numbers and diversity of birds visiting the reserve did not decrease with invasion. This was thought to be due to an increase in habitat types, caused by *C. helmsii* establishment.

Control Attempts

The control of *Crassula helmsii* has generated the greatest volume of literature on the plant, ranging from scientific studies to published reports by land managers. It is likely that funding was targeted towards control based investigations, due to the need for answers. This can be seen in hindsight as a poor use of resources, in that the basic ecology of the plant was and still has been overlooked. There is also no true control practice that exists that is capable of adequately controlling the plant.

Dawson and Warman (1987) propose that any form of control should be adopted, due to the possible development of a monoculture that is thought to be created by *C. helmsii*. They advocate the removal of rare species from areas of invasion, to be replanted at a later date after *C. helmsii* had been removed. They also recommend 'intensive physical removal of the plant and its turf layer'. The use of these types of control is questionable within current management strategies, though they do still occur. Once removed, the issue of what to do with the plant biomass and associated fragment enriched soils and sediments remains an

issue. This is one of the major reasons for not continuing to recommend this method of control.

Leach and Dawson (2000) give a detailed account of the range of control measures that are available for *C. helmsii*. One thought commented on is whether just leaving the plant without interfering with it would actually help in its removal from a site. Failure to establish exists only as anecdotal examples, in garden ponds and two natural ponds within the New Forest, Hampshire (Leach and Dawson, 2000). This is thought to relate to either the continual 'gardening' of pond contents which prevents any species from developing a monoculture, or due to the acidity of the natural ponds (though no comparison of growth related to pH of water had been carried out to support this).

Herbicides

Diquat alginate was studied as an herbicidal control in two pilot investigations (Dawson and Henville, 1991; Spencer-Jones, 1994). Submerged stands were shown to be heavily affected by the chemical, at a dosage rate of 10 litres ha^{-1}. This had the effect of killing the submerged growth forms, but leaving viable floating populations of the plant intact. Though not mentioned, it is likely that the long-term effect of leaving these stands in place would have been later recolonisation of *C. helmsii*. Spencer-Jones also trialled both dichlobenial and terbutryn as control agents, with both showing initial chlorosis effects but eventually little by way of control. Both Dawson and Henville (1991) and Spencer-Jones (1994) concluded that the use of diquat alginate was the most successful future control agent.

A detailed study of herbicidal control was performed, using the three treatment chemicals of diquat alginate, diquat and glyphosate (Child and Spencer-Jones, 1995). The results had varying effects. Diquat alginate caused initial die back of lower sections, but with apical tips remaining healthy. A side effect of the treatment was an increase in fragility of the stems, which resulted in free floating masses of plant tissue which were fully viable – thus encouraging dispersal and spread.

A repeated application of diquat showed even less evidence of control, with no apparent effects upon either rooted submerged, emergent or free floating plant tissue. A further side effect was that of severe damage to the native riparian community (*Iris, Sparganium* and *Juncus* sp.). Glyphosate treatment showed positive results, with emergent plant material displaying signs of chlorosis when compared to control stands of vegetation. Submerged

growth remained viable, which acted as a source of regrowth to replace the effected emerged material. A follow up examination showed growth returning in all areas, though at a slower rate and less sexually developed (flowering time) than the control plots. Dichlobenil granules were also investigated in this study, but results were not reported as the effects were seen to be negligible (though thought to be due to possible affects by heavy rainfall after application).

This study would seem to contradict earlier studies promoting the use of diquat alginate (Dawson and Henville, 1991; Spencer-Jones, 1994), but multiple issues are raised by Child and Spencer-Jones (1995). These included dilution due to environmental factors and the inability to carry out follow up treatments when desired. Though no direct recommendations are given, the conclusion of the paper encourages integrated control attempts with glyphosate for emergent stands and diquat alginate for submerged material.

Dawson (1995) examined both tank and field trial systems. The tank trials consisted of cut turves, taken from field location and maintained using a chalk rich tap water and added fertiliser mix (8-4-4 NPK). Filed trials were selected on the basis of uniformity and plant growth extent, along with 'relevant permissions being available'.

The experiments were subdivided into low biomass and high biomass stands, in an attempt to better understand in-field situations that might be encountered (Spencer-Jones, 1994; Child and Spencer-Jones, 1995). The chemical treatments used were:-

For emergent stands:-

- Asulam
- 2,4-D amine,
- Dalapon
- Glyphosate 'Roundup'

For submerged stands:-

- Dichlobenil
- Diquat 'Reglone'
- Diquat alginate 'Midstream'
- Terbutryn

The results were gained by measuring weight change of the turves in the tank trials. The low biomass submerged trials (3-5 kg fresh weight) were most effectively killed by diquat and

diquat alginate. Dichlobenil and terbutryn were slower, less effective and had the side-effect of an increase in fragmentation caused by an increased brittleness of the stems.

Emergent tank trials showed more complex results. The glyphosate seemed to initially give the best results, but also appeared to slow the growth as opposed to kill it. This resulted in it being the least effective, with diquat showing reductions of biomass that were 3 times more effective.

In high biomass tank trials diquat and glyphosate were applied at higher dose rates than would be applicable in field systems (x50 greater than recommended dose rates). Though greater returns of dead material were found by increasing dose rates, the material still remained viable even after light exclusion by black polythene for 2 months. Field trials were deemed inconclusive due to uncontrolled allocthonous inputs of both water and plant material. The creation of an 'herbicide cocktail', with the possibility of synergism between chemicals, was found not to show significant results.

The conclusions state, in agreement with previous papers, that diquat/diquat alginate is the most effective herbicide for submerged stands, with glyphosate being the most (but not fully) effective for emergent stands. They also comment on the need for flexibility in control, with different sites that show varying levels of colonisation requiring manipulation of dose rates that may exceed recommended permitted levels. The follow up applications that are stated desirable in previous studies (Child and Spencer-Jones, 1995) are questioned in this study (Dawson, 1996), when considering the impact on natives species that are required to occupy the niche created by intense spraying and subsequent progressive contraction of *C. helmsii* growth.

An attempt at control through spraying was carried out by the Royal Society for the Protection of Birds (RSPB) at the Dungeness reserve on the south coast of Kent, England (Gomes, 2005). A diquat based herbicide was utilised for the ditches (submerged stands) with treatments in 2001 and 2002. Application was carried out in late summer. Emerged marginal stands were treated with glyphosate at the same time as the spraying of the ditches. An estimated kill of 70% was achieved in both the submerged and emergent forms (Gomes, 2005). The follow-up treatments in 2002 were deemed 'partially successful', but with re-growth occurring annually between the years of 2002-2005. The current situation at Dungeness is known to be of a wide spread growth of *C. helmsii* across a number of the water bodies. It would seem therefore that

the attempt at control noted by Gomes (2005) had little impact on its progression across the reserve.

A derivative of diquat is still available for use, under the trade name 'Reglone' (HSE, 2013). This was the recommended method of control for both medium areas (20-1000m2) and large areas (>1000m^2) (Leach and Dawson, 1999). However, the ability to gain a licence for its use would seem unlikely, due to the adverse effects upon native flora and fauna (Newman, 2013).

Hydrogen Peroxide

Hydrogen peroxide has been investigated for use in a number of studies (Dawson and Henville, 1991; Dawson, 1996; Leach and Dawson, 1999). Though attractive as method of control due to its non-toxic breakdown products of oxygen and water, it has shown limited success with scorching of the uppermost canopy foliage the only visible effect.

Liquid Nitrogen

Liquid nitrogen was also trialled as a method of contact chemical control, with the same subsequent benefits of having minimal pollution input into treated systems (Leach and Dawson, 1999). The trial, at Holmsley Gravel Pits in the New Forest, Hampshire, England, was carried out in a joint project by The Hampshire Wildlife Trust and BOC Gases. Though initially considered a success, the thick layers of growth prevented direct contact of the liquid nitrogen to the underlying plant material, which was found to prevent adequate control through lack of contact freezing the tissues. The practical application of this method was also found to be difficult, with trained operators and the distance from equipment preventing ease of use (Leach and Dawson, 2000).

Hot Foam

As with hydrogen peroxide, an advantage sought for chemical treatment is the use of a product that leaves little or no residue. One such method, used as a treatment by the RSPB on Old Moor reserve, South Yorkshire, England, is hot foam, called 'Waipuna'. This is a fully biodegradable product, made from coconut and corn sugars which contains large amounts of residual heat and can be used to break down the cellular structure of *C. helmsii* (Bridge, 2005).

When compared to the use of a glyphosate spray however, little differentiation could be found. Both had minimal impact on non-target species, with both achieving a kill rate of approximately 50% of the plant stand (Bridge, 2005). From these results it may be argued that hot foam treatments should replace that of true herbicides as the results are similar, though hot foam is only effective for management of emergent stands.

Flame Throwers

The use of flame throwers as a management tool has also been investigated (Leach and Dawson, 1999; 2000). This has immediate disadvantage as submerged vegetation was not treatable. Subsequent analysis of emerged treated areas was found to show an ineffective level of heat production to kill the roots, and thus the method was abandoned.

Physical Removal

Physical removal was initially thought to be a beneficial control measure (Dawson and Warman, 1987) and is noted as the predominant method of control in Germany (Hussner, 2009). These practices are carried out in the UK by the Internal Drainage Board (IDB), at local authority level, and the Environment Agency, but are predominantly driven more by flood defence work through the removal of biomass than in the prevention of spread and subsequent loss of diversity (Leach and Dawson, 2000). Due to the fragmentation and ease of dispersal, and then re-colonisation through nodal growth, removal would seem to be more about limiting spread than overall control.

One example does exist where removal did result in eradication. This was carried out at Formby Dune System, Southport, Lancashire, which was being managed actively due to concerns for *Bufo calamita* (Natterjack Toad). The control measure involved removing the humic layer of the pond, resulting in the prevention of recolonisation after 12 months. The fate of the removed spoil (and thus *C. helmsii* fragments) was not mentioned (Leach and Dawson, 2000).

Shading

Shading as a form of control of aquatic vegetation was first mentioned in the mid 1980's, when the first research into *C. helmsii* was being carried out (Dawson, 1986). A thick, opaque material that is non-degradable by light and heat (tarpaulin, geotextile, black polythene etc.) is laid out horizontally to cover the plant material. The aim is similar to that used on terrestrial weeds in that if light is excluded, the plant will subsequently die. Though this is true for aquatic macrophytes as well, the same issues surround its use. It is a non-target specific method of control, and so anything covered will be killed. It is also only applicable on a small scale, with estimates of practical usage of 200m^2 for marginal/submerged stands and 400m^2 for terrestrial 'turf' growth (Leach and Dawson, 2000). Certain issues are solely related to the aquatic environment however, such as the dexoygenation of water bodies, which occurs when large stands of plant material are killed in this manner.

Covering of *C. helmsii* with black polythene was used by Bridge (2005), in conjunction with smothering with approximately a 1m depth of soil, on Old Moor reserve, South Yorkshire, England. This was also successful, but not recommended by Bridge due to the small scale, large amount of time and effort required, and disturbance of native species that was created through its use.

Shading with black polythene was utilised by the RSPB on The Lodge Reserve, Bedfordshire, England. *C. helmsii* had been present on the site for approximately 13 years, with treatment being carried out in 2003 (Wilton-Jones, 2005). Coverage was carried out during the winter months of 2003/4, and left in place for six months. Though at the time it appeared eradication had been successful, in a follow up survey in April 2005, it was found to be re-colonising the cleared areas, from the marginal areas that were not covered (Wilton-Jones, 2005). In this instance, it may be that the recolonisation of native species was not rapid enough to counter the re-invasion by *C. helmsii*.

An additional example of shading control was carried out at Hothfield Heathlands, Ashford, Kent. A small area of acid bog within a heathland was treated using black polythene as a shading material in Oct 2010 (Fig 1.9). This was left in place until July 2011, after which the polythene was realigned to cover spread beyond the initial control perimeter. The polythene was removed in July 2012 after continued re-checking for growth, after almost 2 years of in being in place (Rickards, 2013). This is in excess of the prescribed guidelines for control of 8 weeks (Dawson, 1986), which illustrates the naivety of earlier estimates. Whilst no presence of

C. helmsii was found at this location, it was found at a new location further down the bog system, and so may have resulted in fragmentation and further distribution of propagules.

Figure 1.9. An attempt at shading control management, at Hothfield Heathland near Ashford, Kent, taken in April 2010 (Rickards, 2013).

Salt Water

A successful control program was carried out by the RSPB (Charlton *et* al., 2010). This occurred at Hall Marshes in Essex, South-East England. This was a large scale project, which successfully eradicated *C. helmsii* from 120 ha of grazing marsh, by using inundation by salt water. Salt water has been shown to causes necrosis of the tissues in *C. helmsii*, with a negative correlation of increased NaCl concentrations against node regeneration (Smith, unpublished work). Charlton *et al.* (2010) found similar results in field systems. Initial trials found that brackish water allowed similar regrowth potential to that of freshwater. Sea water (30,000 micro siemens) showed potential for control after 5 months of prolonged treatments resulted

in necrosis of *C. helmsii*. This was incorporated into a small 8ha field trial, with permission being gained from government bodies, including Natural England and The Environment Agency. Further artificial pumping was continued into the summer to ensure high concentrations were retained and ensuring full submergence of the plant material. The water was later extracted, again by artificial pumping, with freshwater being pumped back into the drained site. No *C. helmsii* presence was detected.

This trial was extended to the larger 120ha site. Connectivity of pools was ensured before the site was treated in the same manner as the smaller trial. Seawater submergence was maintained for 12 months. Results were the same as for the initial trials, with repeat surveying in 2009 and 2010 showing no signs of *C. helmsii*.

A further application of salt-water was demonstrated by Hampshire Wildlife Trust at the Keyhaven to Lymington marsh complex (Durnell, 2013). *C. helmsii* was first discovered here in 2007, with rapid colonisation in the following year. A system of saltwater inundation, using artificial pumping, was carried out from neighbouring brackish lagoons. This resulted in the complete removal of *C. helmsii*, along with the non-target macrophytes within the mixed complex. Water conductivity values were taken following the treatments to ascertain the reversion rate from brackish to freshwater conditions, to ascertain whether any long-term effects would be shown (Tab. 1.1).

Table 1.1. Conductivity readings from a salt water treated lagoon after inundation (Adapted from Durnell, 2013).

Date of Measurement	Conductivity Reading (mS cm^{-1})
Aug 2011	46.2
Feb 2012	18.4
Aug 2012	14.0
Feb 2013	1.6

The results show a fairly rapid change to near freshwater readings. A lack of recharge of salt from the soil is present, which was a fear of the management team on conducting this treatment method (Durnell, 2013). A more recent study has shown that a range of salinities from 2-8 ppt have shown to be effective at killing *C. helmsii* (Dean *et al.*, 2013).

One consequence of this method of control is that it is not targeted towards *C. helmsii*. Macrophyte coverage has been shown to be reduced from 75%-30% (Charlton *et al.*, 2010), but with no explanation of how much of this was *C. helmsii* compared to native flora. No mention is given to native species in the further 2 studies (Dean, 2013; Durnell, 2013). Seed banks may also have been affected, thereby damaging a key source of native regeneration for natural succession.

The impacts upon invertebrates were studied, to ascertain whether species losses had occurred (Gardiner and Charlton, 2012). Their result surmised that, when comparing populations in 2006 (pre-treatment) to those in 2011 (after treatments) no significant losses of grasshopper, bush cricket or yellow meadow ants had been found. Ditch samples, taken shortly after the treatments were concluded, showed no significant reduction in aquatic invertebrates. Some rarer macrophytes, such as sea barley *Hordeum marinum* (sea barley) were thought to have benefitted from this method of treatment (Charlton *et al.*, 2010).

Other Control Methods

Leach and Dawson (2000) gave three examples where water turbidity may lead to a reduction in growth. This may be akin to the shading measures already mentioned, but more applicable due to the removal of practical limitations that shading and the laying of material presents. They are only of use to controlling submerged stands, being that the emergent stands would not be affected. These suggestions included:-

- The release of bottom feeding fish, including Tench (*Tinca tinca*) and and/or Carp (*Cyprinus carpio*) to promote sediment disturbance and thus particulate suspension within the water body.
- Nutrient seeding to promote algal growth – ‘intentional’ eutrophication and the subsequent organic detrital/algal growth cycle implementation.
- Application of chemical dyes to ‘darken’ the water body (Fig. 1.10). These methods are currently being tested and as such are not widely available (Newman, 2013). The dyes act by limiting the light available to *C. helmsii*, thereby reducing photosynthetic activity.

Figure 1.10. Blue dye control attempts in ponds in the New Forest National Park (Chatters, 2015).

Newman (2013) investigated the use of dyes, but found them to have limited success. They were also found to cause significant side effects, which included elongation of the plant and increased stem brittleness. Both of these side effects can lead to an increased chance of dispersal.

A comparison of treatments and their effects upon native macrophytes and macro-invertebrates was carried out in the New Forest National Park, UK (Tab. 1.2).

Table 1.2. Summary of control attempt in The New Forest National Park (Adapted from Ewald, 2013).

	Effects upon *C. helmsii* coverage		**Effects upon native plant coverage**
	February 2012	June 2012	June 2012
Herbicide	Decreased by 84%	Returned to pre-treatment stage	No Change
Hot Foam	Decreased by 25%	Increased by 5%	Decreased by 10%
Dye	No Change	No Change	Increased by 10%
Control	No Change	No Change	Increased by 10%

The results of the trial show that no long term strategy is for control is evident, with herbicide treatments and follow-up applications likely to be the only method of providing some level of control, but not eradication. No species loss was recorded in any of the treatments, with some examples of the re-emergence of rarities being recorded (Ewald, 2013).

Biological Control

The use of grass carp (*Ctenopharyngogon idella*) has been attempted, but *C. helmsii* was found to be the least favoured food source. *C. idella* would, therefore, be more likely to remove native species before *C. helmsii*. Anecdotal evidence exists for some grazing of shoot growth by native wildfowl, as well as livestock used upon nature reserves for scrub control – most notably Highland cattle (*Bos taurus*) and Konik Pony (*Equus ferus f. Caballus*). This in itself is not enough to count as true control however, being that the feeding style is more generalist rather than targeted towards *C. helmsii*. Issues surrounding dispersal of fragments by cattle and ponies would also likely be an issue.

The search for a biological control agent is currently being undertaken by The Centre for Agricultural Bioscience International (CABI). A classical biological control agent is being sought, due to its cost effectiveness through the creation of a self-sustaining population and its specificity to the target plant (Varia, 2013). The aim is to source and release an agent as successful as *Stenopelmus rufinasus* (North American Weevil) has been, on the aquatic invasive *Azolla filiculoides* (Floating Water Fern).

A research trip to Australia and Tasmania was conducted in 2010, in an attempt to collect a range of possible control organisms (Varia, 2013). This resulted in the collection of 5 possible pathogens and a range of arthropods which showed some preference for *C. helmsii*.

Follow up trials in the UK have investigated these specimens for either presence of inoculation (pathogens) or presence of laying strategy or life stages (arthropods) upon *C. helmsii*. This was followed by trials upon closely related native plants, such as *Crassula tillaea* (Mossy Stonecrop) and *Crassula aquatic* (Water Pygmyweed), as well as species that share a similar niche such as *Pilularia globuliferaI* (Pillwort).

Initial trials indicated a weevil of the *Steriphus* genus as a likely candidate, due to the damage it caused to *C. helmsii* tissues. The follow up trials indicated that it was not host selective however (Varia, 2013). More recent trials have shown that *Hydrellia perpelxa*, a leaf mining fly, shows host specificity towards *C. helmsii*, with other test species remaining viable. Tested species include *Calitriche spp*. (Water Starworts) and *Potamogeton spp.* (Pond Weeds)(CABI, 2014). To date, 60% of a list of native species has been tested (CABI, 2014). *H.perplexa* also has an aquatic larval stage, which would be beneficial when trying to produce viable populations within the freshwater habitat that *C. helmsii* occupies. An *Aculus spp.* mite has also been identified as feeding on the growing stems of *C. helmsii*, resulting in damage to new growth and a reduction in vigour. 40% of non-target, native species have been tested so far (CABI, 2014).Two pathogen species (*Cercospora* sp. and *Colletotrichum* sp.) have also been identified, with one attacking the leaf whilst the other attacks the stem, resulting in the collapse of the plant (Varia, 2013). The *Colletotrichum* species is currently under evaluation in quarantine (CABI, 2014).

This examples may provide a biological control agent in the future, but due to the length of time required to develop successfully (*Fallopia japonica* – Japanese knot weed has taken 20 years to get to a field trial – Varia, 2013), it is unlikely to be a viable control option for some time.

Literature Overview

C. helmsii has received attention from a range of different researchers with varying interests. A search of peer-reviewed articles, using Web of Science, JSTOR and Google Scholar containing

the search terms '*Crassula helmsii*' (as of March 2015) was conducted. The results of this search were divided thematically (Fig.1.11).

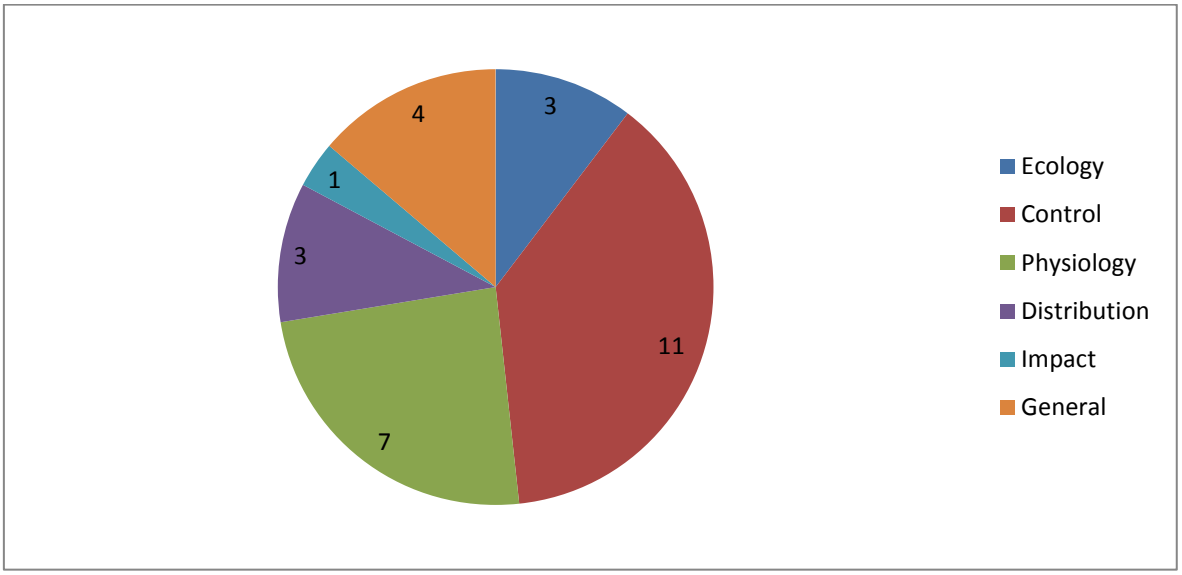

Figure 1.11. The peer-reviewed research of *C. helmsii* divided thematically. The search was conducted using '*Crassula helmsii*' as the search term, using Web of Science, JSTOR and Google Scholar as search engines (correct as of March 2015).

The area of research that has received the least attention is that of the overall impact of invasion on native species. It is for this reason that this area of study was chosen for the main emphasis of this research project, and will be addressed in chapters 3-7. The distribution patterns of *C. helmsii* are also an understudied area in comparison to the more numerous studies on control and physiology. Within these distribution papers, no research has addressed either the overall spread patterns or the distribution of *C. helmsii* on a landscape scale basis. The use of computer modelling and digital maps had also not been considered as a method of investigating its historical spread characteristics. Chapter 2 examines a study that looks at the distribution of *C. helmsii* across the UK. It also considers how its distribution has changed over time and whether a relationship between the location of records and geographical features exists.

Chapter 2 – The Spread of *Crassula helmsii* in the UK and Kent.

Introduction

Understanding how a species spreads allows for more informed predictions of future habitat colonisation and population sizes, as well research driven management decisions to be made. Due to *C. helmsii's* ability to reproduce asexually (Dawson and Warman, 1987; Hussner, 2009), and be well adapted at reproducing from small nodal fragments (Hussner, 2009; Smith, unpublished work) *C. helmsii* is well suited to spread rapidly. Dispersal mechanisms have been studied, with possible vectors including livestock (Dawson and Warman, 1987; Chatters, 2013), wildfowl (Denys *et al*., 2014), the horticultural aquatics trade (Laundon, 1961; Swale and Belcher, 1982; Dawson and Warman, 1987) and mechanical control methods (Hussner, 2009). No attempt at investigating the spread patterns of *C. helmsii*, which takes account of change over time, locality and dispersal distance has previously been attempted.

Early work on species spread patterns by Skellam (1951), were based on the *Ondatra zibethica* (American muskrat). This study led to the concept of the general diffusion model on invasive species spread (Skellam, 1951). This relatively simple model proposed that a species, released from single foci, would spread out at a constant rate of growth, akin to a traveling wave front (Marco *et al*., 2011). A relationship was shown to exist between the square root of the area occupied by the population and a linear increase in time.

Short Distance and Long Distance Dispersal

Distributions are complex and are governed by a range of factors including, but not limited to, life history traits, population processes, habitat suitability and disturbance (Marco *et al*., 2011). Species do not conform to the standard pattern of simple, short distance dispersal mechanisms as originally proposed (Skellam, 1951Higgins *et al*., 2003; Muirhead and Macisaac, 2005). Long distance dispersal, or stratified dispersal, is studied on a macroscopic scale, encompassing multiple habitats across entire countries and beyond national boundaries (Higgins and Richardson, 1999; Higgins *et al*., 2003). Short distance dispersal (SDD) and long distance dispersal (LDD) yield different spatial distributions (Moody and Mack, 1988). SDD events are generally compound with a defined spatial front and few satellites populations (Moody and Mack, 1988). LDD events tend to produce smaller, but more numerous satellite

communities, which will often coalesce over time when satellite communities are no longer produced (Marco *et al*., 2011). LDD tend to show no true movement front (Skellam, 1951; Marco *et al*., 2011). This is a factor that makes LDD species difficult to measure, research and control.

SDD species are more influenced by habitat heterogeneity than LDD species. With *Gledistia triacanthos* (Honey Locust), a SDD favouring species, growth rates fell from 4.4 m yr^{-1} in suitable soil conditions to 1.9 m yr^{-1} in more rocky, unsuitable soils (Marco *et al*., 2011). LDD species counteract these unsuitable conditions by having more satellite communities, which spread by greater dispersal around, rather than through obstacles. They also show less density dependent limitations to growth than SDD species, due to having greater edge habitats than SDD. This would likely lead to a faster growth rate.

Darwin (1878) initially noted the importance of long distance dispersal (LDD), but this has remained a relatively understudied area due to the more easily quantifiable studies of short distance dispersal (SDD). A lack of statistical methods of interpretation of LDD dispersal events, along with the understudied 'rare' dispersal processes through unusual vector movements is stated as one of the causes (Higgins and Richardson, 1999). Both LDD and SDD can occur together, and so a simple model governing the spread of species is generally not applicable (Shigesda *et al*., 1994).

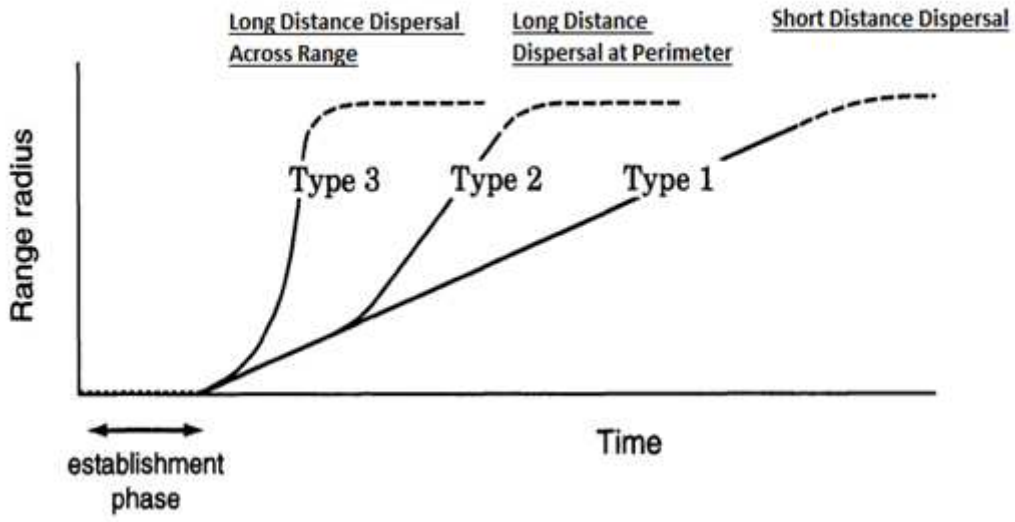

Figure 2.1. Species expansion examples (Adapted from Shigesda et al., 1994).

- Establishment phase – invader may be ill-adapted to new habitat, or that they remain below detectable limits.
- Type 1 tends to remain in close proximity to initial invasion, with 2 and 3 exhibiting LDD.
- Type 2 show LDD only from the fringe of the range and travel moderate distances.
- Type 3 shows LDD across the range, hence accelerating rates of expansion until saturation.

Each model experiences an initial establishment phase, where the invasive is spreading only by SDD and so is not escaping from its initial point of invasion (Fig. 2.1). An example of this is the release of *Sturnus vulgaris (*European Starling) in New York, which took 10 years to establish before range expansion occurred (Shigesda *et al*., 1994). The most rapid spread is shown by the Type 3 species, where LDD is occurring across the full range of the habitat and not just fringe areas, and therefore creates the largest number of subsequent nascent foci beyond the initial invasion site.

Modelling Species Spread

The aim of modelling spread dynamics is to enable predictions to be made on the movement of species. For non-native species, it allows advance warning of possible potential impacts (Weber and Gut, 2004). One way in which this is carried out is in by using databases. These provide information on habitat preferences, species biology and likely methods of introduction, like possible vectors and pathways (Verloove, 2010). Decision trees can be used in conjunction with databases, which give species a specific risk category (Reichard and Hamilton, 1997). Risk must be assessed, which takes account of the loss of native species, to ascertain whether a detrimental impact occurs (Hiebert and Stubbendieck, 1993). Often this requires not just knowledge of spread, but accurate initial identification. If not accurate, practices may be put in place that could lead to further colonisation and subsequent population increase (Verloove, 2010). Using accurate models can also aid in species distribution predictions. Weber and Gut (2004) achieved an accuracy of 76.6% with their model, which looked to identify invasive species that established. The models took account of high ecological and taxonomic diversity, lack of ecological data and variation in invasiveness within the range of species studied.

Satellite communities and their importance in invasive spread were studied by Mack and Moody (1988). Early in an invasion, fewer large satellite communities are the main location of the invasive species, which illustrates the establishment phase (Fig. 2.1 (Shigesda *et al*., 1994)). If dispersal is not via LDD, but still escapes the confines of the original parent community, than a secondary model is proposed, called the 'coalescing colony model' (Fig. 2.2). Though not the same as Skellam's (1951) original diffusion model, the overall results are similar, with new colonies only dispersing locally. These soon become engulfed by the parent community as it enlarges. Loss of edge effect and the subsequent limitations to dispersal also occur.

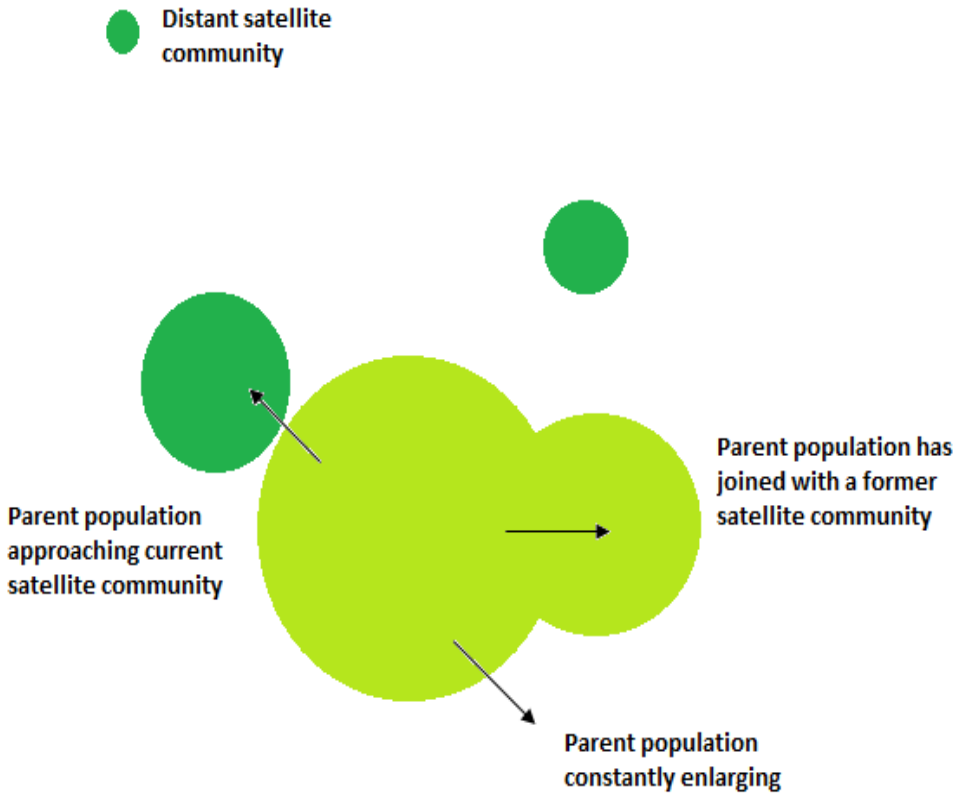

Figure 2.2. The Coalescing Colony Model (adapted from Moody and Mack, 1988). Localised satellite colonies are soon joined to the parent population.

Smaller satellite colonies which establish by LDD rapidly occupy more area than the original parent community, which in itself is expanding by SDD. This illustrates another form of species spread called the 'scattered colony model' (Fig. 2.3). The initial parent satellite focus loses its importance for propagule creation and delivery, and will remain ineffective unless the satellite foci are lost (through such factors as control, major disturbance and disease). The smaller

satellite foci expand at a greater rate when compared to the parent foci. This occurs due to less density dependant competition and the ability for more propagules to reach the 'front-lines' due to a greater 'edge-effect' (Moody and Mack, 1988; Marco *et al*., 2011).

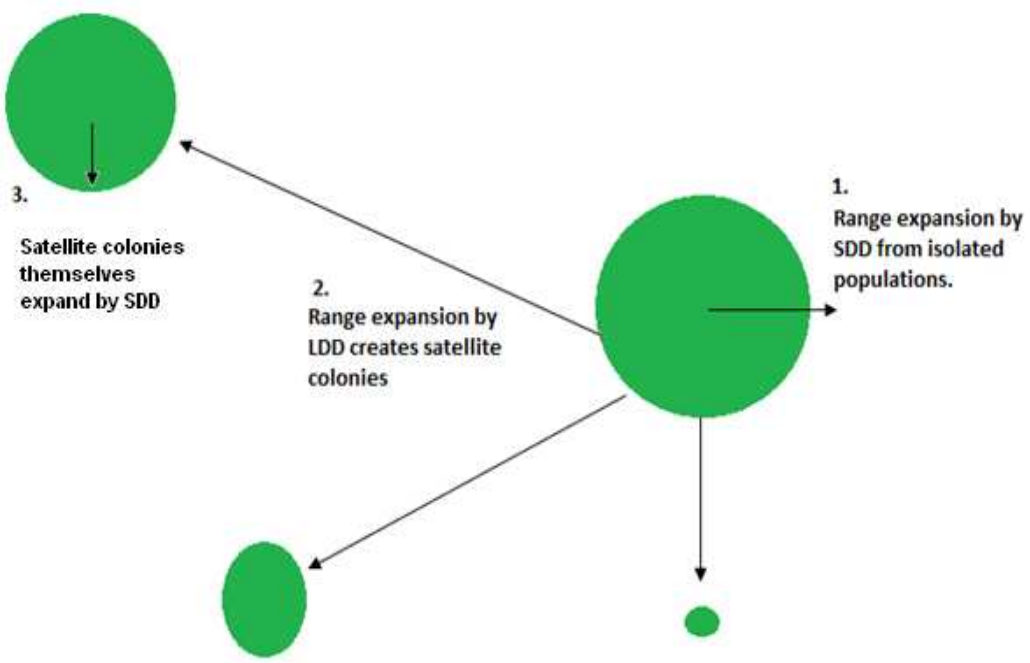

Figure 2.3. The Scattered Colony Model (adapted from Moody and Mack, 1988). Satellite colonies spread further from the parent colonies, with all populations spreading by SDD.

Most early models conform only to homogenous landscapes, due to the difficulty of modelling a heterogeneous and highly diverse system in an adequate way (Higgins and Richardson, 1999). More recent attempts have been made to model both SDD and LDD together as more sophisticated modelling software has been developed (Gilbert *et al*., 2004; Giloli *et al*., 2013). These studies indicated that often both mechanisms are operating together, and so support the earlier ideas of the 'Scattered Colony Model' (Moody and Mack, 1988).

Muirhead and Macisaac (2005) used inland lakes as method of modelling LDD. Lakes provide a source of landscape wide heterogeneity as they are isolated from each other, and have different habitats between them. They identified that the major factor that influenced spread was the degree of connectivity between them, which in their example was the frequency of

human mediated boat traffic. The more highly connected the lake was (those accessed by human traffic more frequently) were found to show the greatest level of non-native species. The results also illustrated how clusters of invaded lakes emanated from a central nascent population. Due to the inability for them to combine (due to the heterogonous nature of the habitat), it acts as a good example of the scattered colony model. A further lake study, examining *Dreissena polymorpha* (Zebra Mussel) was also shown to support the scattered colony model (Herbet *et al*., 1989). Examples of the scattered colony model are not limited to national boundaries. Ruiz *et al*. (2000) described the use of shipping vectors and the colonisation of coastal fringe habitats, by invasive species as a method of international spread. The satellite colonies, that spread from the initial parent colony located overseas, would become the primary parent foci in the new country. From these populations, further satellite colonies could then be generated through LDD.

Management Informed Models

Fragmentation of habitat as a control strategy for SDD species may be a potential management tool to prevent spread. The invasive species would need to be accurately identified as a SDD favouring species before this was carried out, due to the increase in edge perimeters it would create. This would actually enhance the spread of LDD species (Fig. 2.4). The consequence of making the wrong decision at this point could lead to an acceleration of invasive species spread.

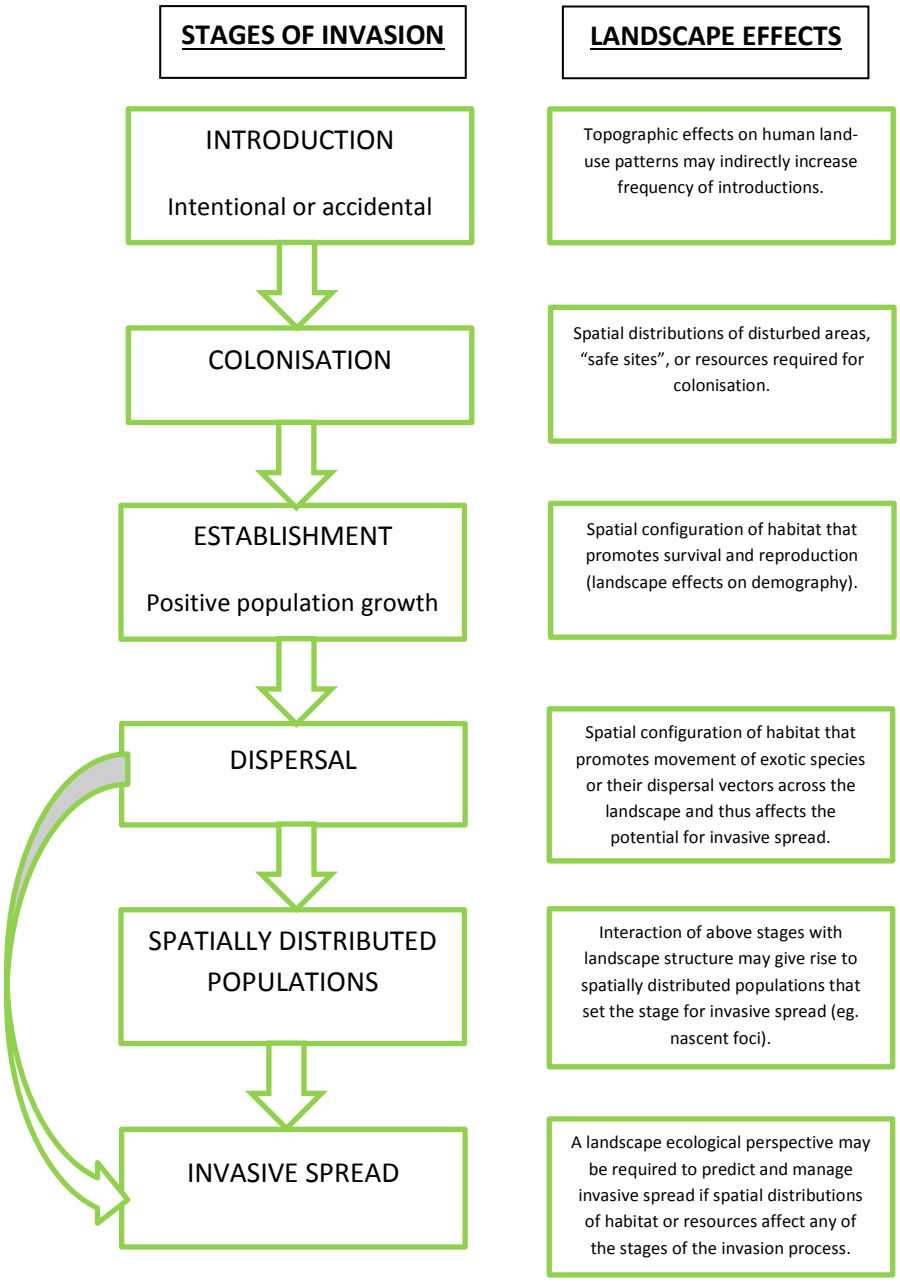

Figure 2.4. The stages of invasion in conjunction with how landscapes are altered during invasion (Redrawn from With, 2001).

By studying spread at larger scales, management decisions can be made that may be more successful at preventing wide scale colonisation and dispersal of invasives. An example of this could be the installation of habitat breaks, where habitats are intentionally fragmented, in an attempt to prevent the spread of species that have limited mobility across unfavourable habitats – akin to fire control by the use of cutting fire breaks (With, 2004.) Fragmentation also

acts against current species conservation theory of looking to join habitats and promote 'native 'species spread (Ghergel and Papes, 2015). It also assumes that no vector is present, which would allow transmittance across unfavourable habitats, even if the species in question does not possess the required adaptations.

Edge effects have been demonstrated in woodland habitats, which have been shown to contain more non-native species than the interior woodland habitat (Brothers and Spingarn, 1992). Invasion by *Pittisporum undulatum* is found to be enhanced along the edges of scleropyhll bushland in Australia (With, 2001). Management that minimises edge fragmentation of native habitats was found to reduce edge effects and decrease the potential for biotic exchange between habitats (With, 2004).

Sources of data for invasive species are an important tool for management, as it is unlikely that land managers would have access directly to peer-reviewed articles. The accuracy of the literature accessible to land managers is therefore important. A study of Czech data for the Flora Europeae catalogue was found to be erroneous (Verloove, 2010). These problems, along with poor identification skills, can lead to the establishment of invaders through 'disguise' – hidden by similar native species. The species that follow are examples of this.

- *Digitaria violascens* – a crabgrass, looking very similar to the native European *Digitaria ischaemum*. Benefits from management practices of creating open, semi-natural vegetation.
- *Eleocharis pellucid*a – rush species, looking similar to the Italian native *Eleocharis carniolica* (which is very rare and a target species). Both species grow sympatrically, so efforts to encourage/kill will affect the other.
- *Juncus dichotomus* – similar to *Juncus tenuis*, one of the most widespread xenophytes in Europe. *J.dichotomus* tends to be more specialised, being confined to temporarily wet habitats – though this has not helped in aiding identification from the native. (Verloove, 2010).

Knowing the spread dynamics of a particular species may enable more targeted control methods to be employed. Control strategies may also be influenced by more direct practical issues. These include time, money labour and skills (Wilgen *et al*., 2001). Due to the stochastic nature of invasion, and the lack of data and literature that exists around individual species, a trial and error approach is likely to remain the method most utilised.

Vectors

Human mediated vectors are often the cause of LDD of invasive species (Moody and Mack, 2001). It is thought that over 70% of naturalised exotic species in Australia were intentionally introduced as ornamental species by humans (Weber and Gut, 2004). Humans provide an effective vector path for LDD (Higgins *et al.*, 2003), be it through direct or indirect methods. Natural vectors such as the translocation of seeds by bats, and the adherence of seeds, propagules and molluscs on the feed of birds are also important delivery methods (Higgins *et al.*, 2003). It is estimated that 21.4% of the plant species that dispersed to Easter Island did so in mud on the feet of birds (Carlquist, 1967). Though morphology of propagules may aid SDD effects, it has been shown that morphology is not directly linked to LDD, and so numbering and evaluating dispersal mechanisms over longer distances is difficult. Darwin originally studied the adherence of molluscs to bird's feet, using the legs of deceased wildfowl, which had been colonised by snails to investigate adherence abilities (Figuerola and Green, 2002). The results gave rise to initial considerations of how important water birds are at dispersal between water bodies, and thus are able to implement LDD over heterogeneous habitats between water bodies. These results have been supported with discoveries that the genetic distance and geographical distance have been shown to be related in *Potamogeton pectinatus* (fennel pondweed) (Mader *et al*., 1998), whilst the genetic distribution of the bryozan *Cristatella mucedo* in northern Europe follows the major waterfowl flyway (Freeland *et al*., 2000).

Use of Geographical Information Systems

The development of geographical information systems (GIS) has enabled relationships to be studied between species spread and geographic variables. The spatial relationship between *Agrilus planipennis* (Emerald Ash Borer) and its distribution was investigated using ArcGIS computer software (Muirhead *et al*., 2006). This investigation allowed future colonisation to be predicted. In a similar way, diseases have been mapped, with the output being used to generate not only projected spread patterns, but possible vectors and methods of transmission (East *et al*., 2008). By using geographical modelling software, large datasets can be analysed quickly and efficiently. When joined with additional mapped features such as roads, rivers, climate and urban population centres, a greater level of understanding can be gained as to where species have spread over time. This may allow for interpretations to be made as to why species spread to some areas but not others.

Aims

Crassula helmsii was first recorded in Essex, England in a naturalised habitat in 1956 (Laundon, 1961). Initial concerns were that it would grow rapidly, colonising habitats and causing widespread loss of diversity of native species, through the establishment of a monoculture. To be able to interpret whether *C. helmsii* had spread across the country in this way, a series of computer based studies were constructed. These attempted to determine whether propagules spread by SDD or LDD, or a combination of the two. Differences between land designations were also investigated. This was in an attempt to discover whether the level of management or the overall aims of a site were responsible for differences in *C. helmsii* colonisation. An attempt to find whether spread was due to the horticultural trade (as the plant was known to be spread in this way) or by another method was also carried out.

Method

Data Sources

The National Biodiversity Network provides a database of records (National Biodiversity Network, 2014). It is monitored by the Centre for Ecology and Hydrology and the Joint Nature Conservation Committee. A large number of organisations provide data to the database, which is then accessible in the form of maps and raw data in a number of formats.

The Botanical Society of Britain and Ireland (BSBI) provide plant-based datasets. These are available in hectad (10 km) or tetrad (2 km) level divisions for species, dependent on the accuracy of the initial records. On correspondence with the BSBI, specific data sets and raw data were requested (Lockton, 2012; 2013).

This provided 3 data sets:-

- A full UK dataset from the NBN Gateway database of 904 records.
- A full UK dataset from the BSBI of 4461 records.
- A smaller dataset for Kent at county level with site specific locations from the BSBI of 122 records.

Distribution Method – SDD vs. LDD using UK Data

In order to evaluate whether *C. helmsii* has spread by SDD or LDD, the data sets were grouped into decadal bands. These were added to a standard map interface on MapInfo mapping software, using a standard Ordnance Survey base layer and British Geological Bedrock layer (Fig. 2.5) to confirm the coordinate system. The coordinate system used was the British National Grid [EPSG: 27700].

To ensure that both the grid systems for the base layers and the data points were aligned, the datasets were transformed into X and Y (Easting and Northing) coordinates. Once applied to the 2 base layers, a manual check of randomly-selected sites was carried out to confirm that the data was aligned properly. Once plotted, this gave 6 separate layers of data for analysis.

- 1950-1959
- 1960-1969
- 1970-1979
- 1980-1989
- 1990-1999
- 2000-2009

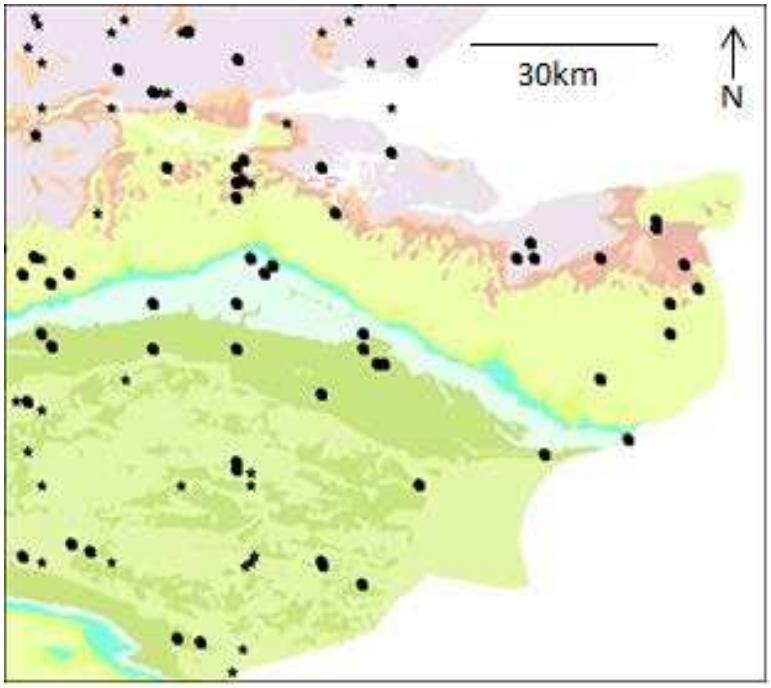

Figure 2.5. *C. helmsii* location records plotted onto the British Geological Bedrock layer. In this example, 1980-1989 datasets are represented by stars, whilst 1990-1999 is represented by circles. The area shown is the south east of England.

In order to assess spread patterns, buffer zones were created around all data sets excluding the 2000-2009 layers. Buffers of 5, 10, 15 and 20km were created around each individual plot, which resulted in four buffer zones around each data point (Fig 2.6). A query function was constructed, which allowed for data to be measured as either present or absent within each buffer. By keeping each data layer and buffer separate, the query could be used to isolate individual decadal increases within each of the buffer zones.

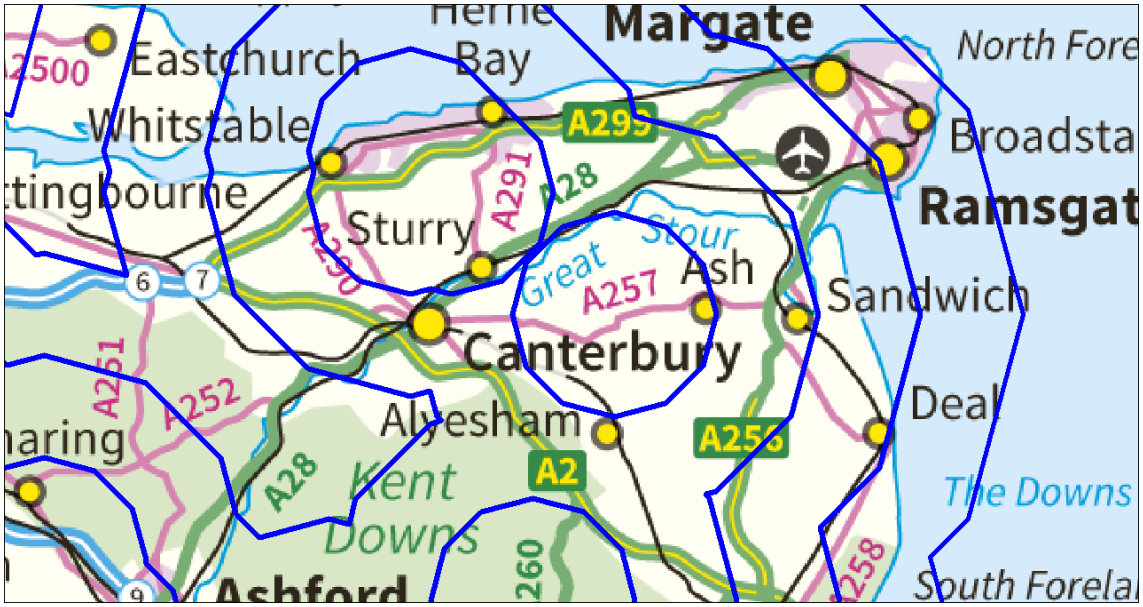

Figure 2.6. Buffer zones created around data points. In this example, the different buffers of 5, 10, 15 and 20km are shown surrounding a record of *C. helmsii*. The base layer shows the standard Ordnance survey layer used to manually check layer alignment.

1950-1959 data was buffered by the 4 distance buffers and analysed for the presence of 1960-1969 data. This returned 4 values. Subsequent data was queried in the same way, with each buffered data set being queried by the following decadal year group's data. These values were divided by the original total values to give a percentage score of total number of *C. helmsii* records, and plotted onto line graphs. Both the full NBN data and BSBI data sets were analysed in this manner.

Designated Sites using UK Data

C. helmsii records were compared with the following site designations:-

- National Nature Reserves (NNR)

- Local Nature Reserves (LNR)
- Sites of Special Scientific Interest (SSSI)
- Areas of Outstanding Natural Beauty (AONB)
- Country Park (CP)
- Special Areas of Conservation (SAC)

Where sites were categorised as more than one of these, analysis was not adjusted and both designations were used. The designated sites were overlaid onto an Ordnance Survey and British Geological base layer, to enable accurate cross checking. Coordinate system projections were as before. The mapping layers for the designated sites were provided by the Natural England GIS Digital Boundary Datasets (Natural England, 2013). When entered in to the mapping project, the sites were cross checked for projection alignment by studying known sites for accuracy. A boundary layer of 2km was created around each designated site. This resulted in a further 6 mapping layers, which were coded:-

- NNR +2
- LNR +2
- SSSI +2
- AONB +2
- CP +2
- SAC +2

In total, 12 map layers were available, excluding the base layers used for reference (Fig. 2.7).

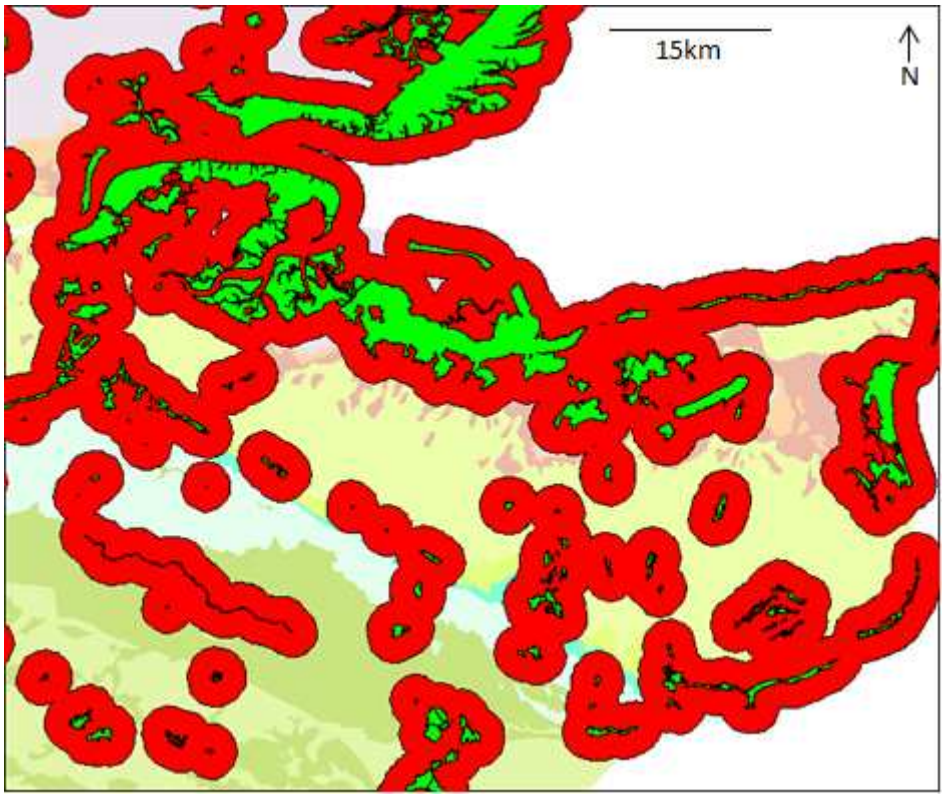

Figure 2.7. SSSI designated sites (green) and the 2km buffer zones (red), plotted onto an outline geology bedrock layer. The buffer zones were subsequently scaled in the analysis, due to the increased size when compared to the designated sites.

The *C. helmsii* data records were divided into decadal bands in the same way as for previous analyses. Query functions were constructed to determine the presence of *C. helmsii* records within the designated site layers, and the accompanying 2km buffer zones. To enable the results to be scaled (as the buffers were larger than the designated sites from which they were generated), the total buffer size was subtracted from the initial designated site layer. Percentage presence was determined by dividing the number of records of *C. helmsii* by the designated site total size or buffer total size. Statistical analysis was carried out using a Friedmann repeated measures test, with a comparative line graph for visual distinction. Both variables were compared (years and sites) to enable two way comparison. This was carried out for both NBN and BSBI datasets.

County Level Analysis Using Kent Data

The Kent dataset had a minimum accuracy to tetrad level (2 km^2). A total of 122 records were available for analysis. The data was transformed into Eastings and Northings, to allow for cross compatibility with the map layers. The transformed data was added to a mapping project on MapInfo, along with the standardised Ordnance Survey and British Geological layers (Fig 2.8).

Figure 2.8. The county level Kent data set (green pins) plotted onto the base layer of Ordnance Survey and British Geological layers. (Throughout this study, using these two layers was found to aid in the confirmation of projection systems and in manually checking the *C. helmsii* record locations for accuracy).

The projection used was the same as in the previous analysis. Additional geographical features were added to the data points:-

- A-Roads
- B-Roads
- Minor Roads
- Motorways
- Railways
- Settlements
- Rivers
- Woodlands
- Coastlines

- Lakes

The geographical datasets were accessed via the Ordnance Survey Open Data request (Ordnance Survey, 2013) and overlaid onto the existing mapping layers (Fig 2.9).

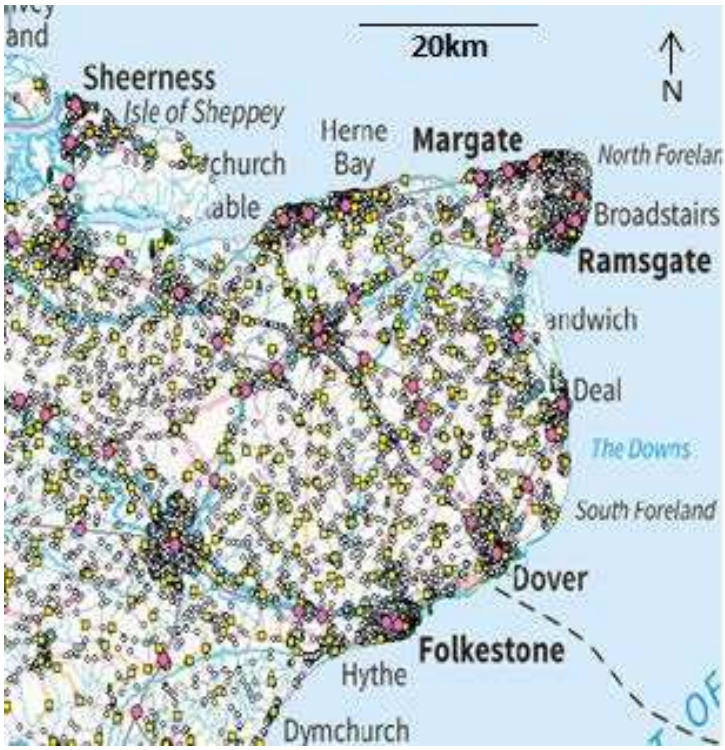

Figure 2.9. Geographical data overlaid onto an ordnance survey base layer. The large number of features plotted on the GIS maps allowed for a range of comparisons. In this image, the red circles can be easily seen, which indicate railway stations.

Distance calculations were performed using MapInfo tools, with each *C. helmsii* record measured to its nearest geographic feature (Fig. 2.10). This enabled a range of measurements to be calculated for each record, with the subsequent data analysed using regression analysis.

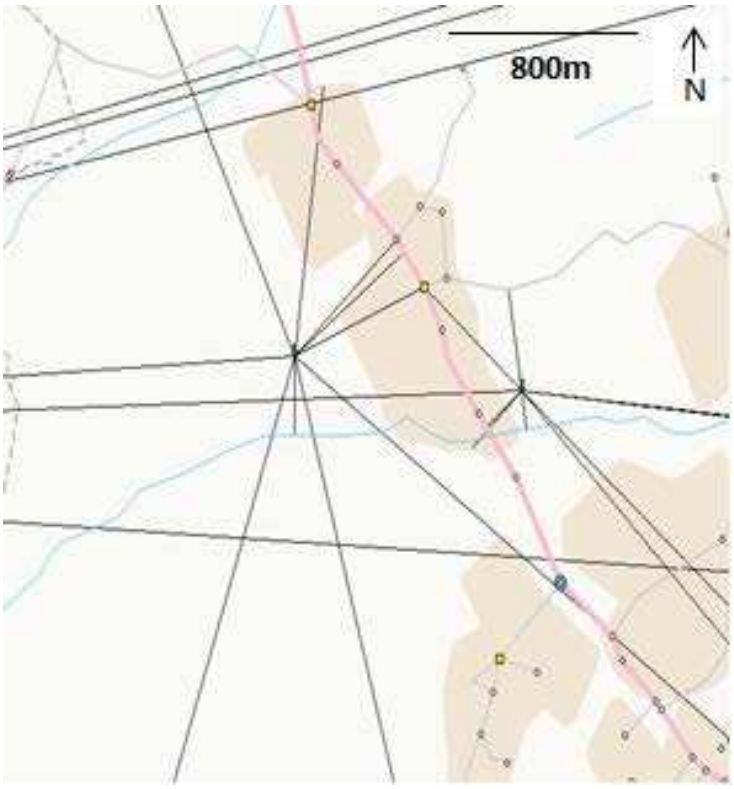

Figure 2.10. Distance measurements (straight lines) from *C. helmsii* records (green pins) to the nearest geographical features that were added to the project. This image is a magnified view of Blean Woods National Nature Reserve, to the north of Canterbury, Kent. A minor road (red line) and waterway (blue line) can be seen in this image.

Results

Records Overview of UK Data

The cumulative data show a progression in the number of cases up until the late 1980's, where a sudden spike in records occurs (Fig. 2.11). After this date, the cumulative results continue to rise at a similar gradient. The yearly records also show a peak in the late 1980's. Fluctuations occur after this but with no true definitive patterns seemingly apparent. The decadal grouping of data portrays a decreasing number of records since the 1980's peak, with a steady downwards progression in records. The 5-yearly bandings of records also appear to support these findings.

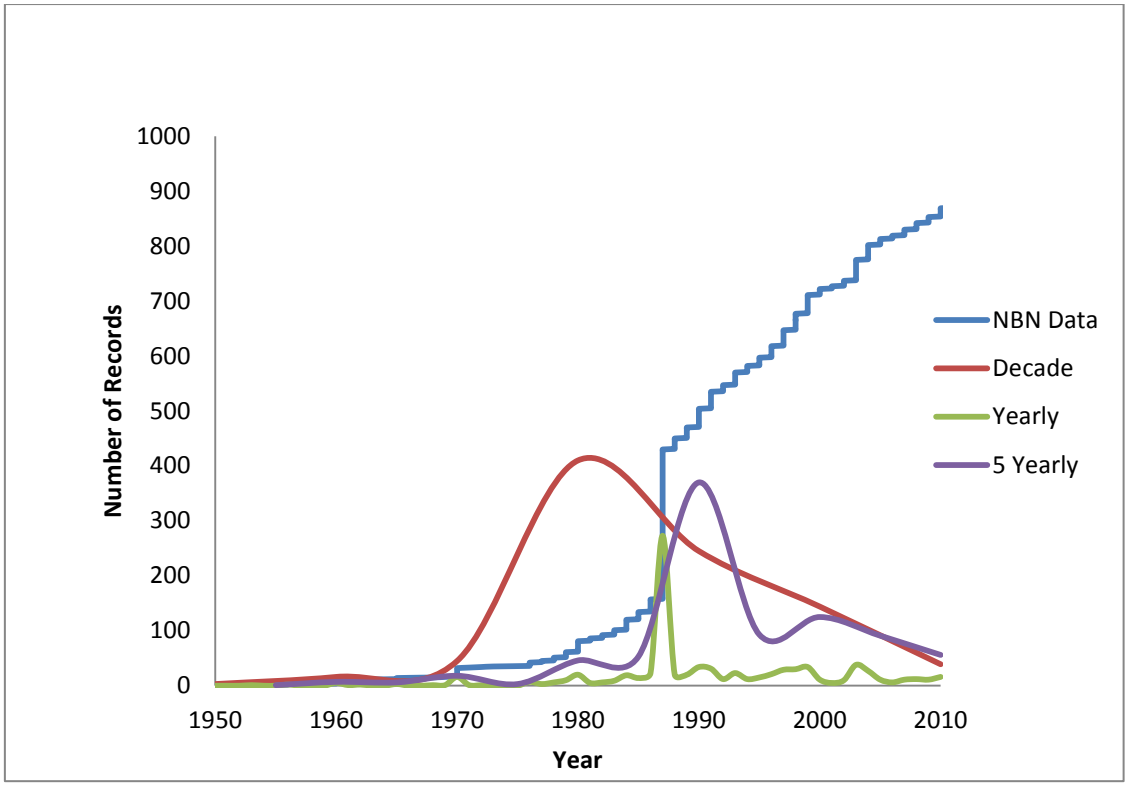

Figure 2.11. Comparison of cumulative and date banded records of *C. helmsii* from the NBN Gateway database. The data is shown as a cumulative set, yearly groupings, 5-year groupings and 10 year groupings.

The BSBI dataset (Fig. 2.12) was treated in the same manner as the NBN data set (Fig. 2.11). Cumulative recordings show 2 spikes in data, in the late 1980's and 2000. Yearly values mirror these 2 peaks, with surrounding data showing a steady number of records. The decadal subdivision shows 2 distinct rises in data, which can be attributed to these aforementioned increases in data records, with a steep decline towards 2010. The five year sub-division shows evidence for these peaks, but does not display the steep decline in records as clearly as the decadal grouping.

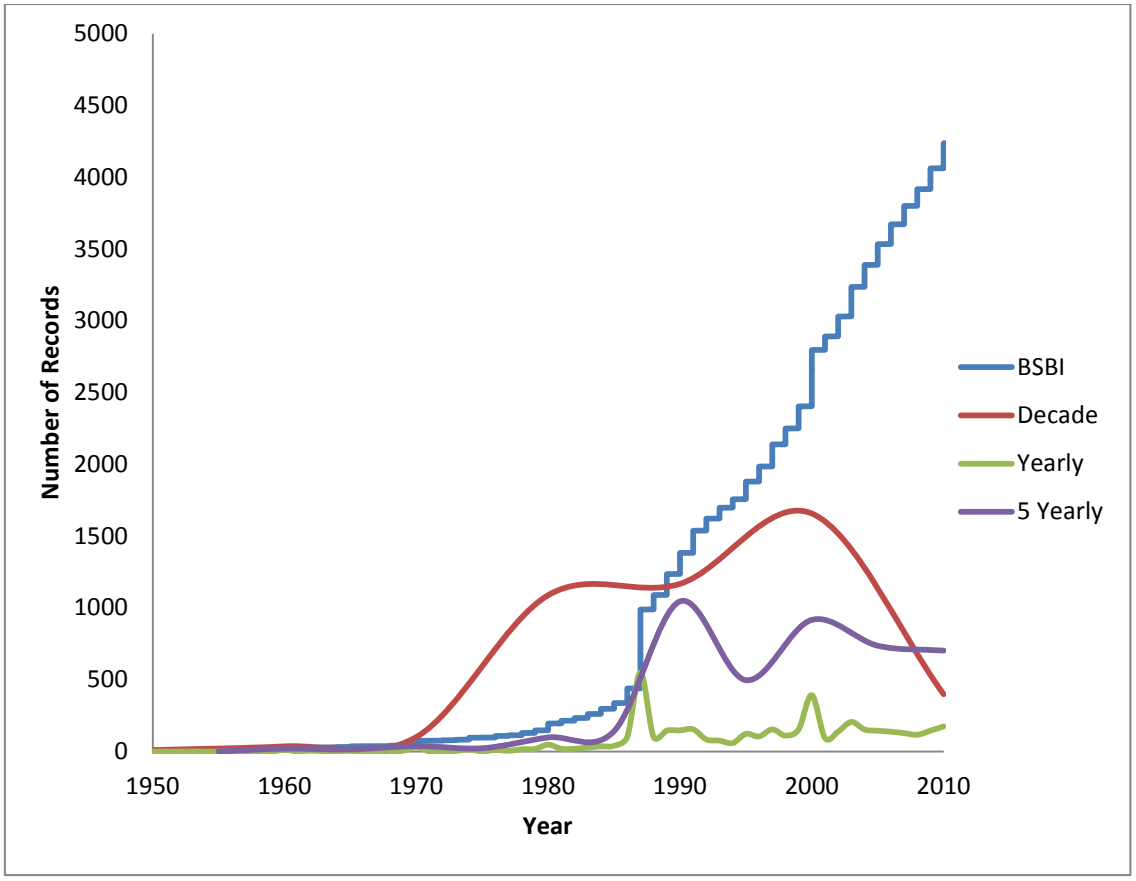

Figure 2.12. Comparison of cumulative and date banded location records of *Crassula helmsii* from the BSBI database. The data is shown as a cumulative set, yearly groupings, 5-year groupings and 10 year groupings.

Distribution Method Using UK Data

The NBN dataset (Fig. 2.13) showed a similar trend across all distance buffers between the years 1950-1980. Between 1980-1990, the number of records rises sharply, with all buffer zones reflecting this increase. The 5 km buffer shows a smaller rise, from 0% in 1950 to 13.57% in 1990. The largest rise was shown by the 20 km buffer results, which rose from 30.24% in 1980 to 83.67% in 1990. All data sets showed a decline after this peak, for the subsequent 2 decades of recordings.

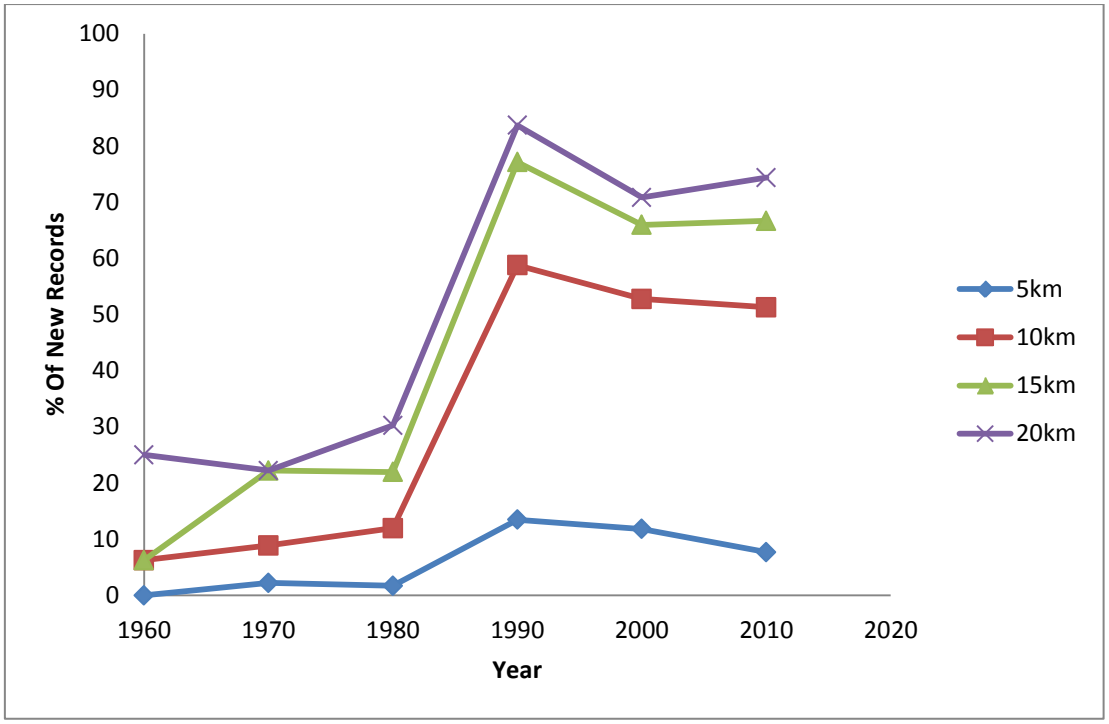

Figure 2.13. Percentage of new records of *Crassula helmsii* falling within buffer zones of previous years records (NBN data). The four buffer zones are 5, 10, 15 and 20km and are shown independently.

The BSBI analysis (Fig. 2.14) showed similar results to that of the NBN set (Fig. 2.13). A sudden rise in the percentage of records, falling within the subsequent decade's buffer, was again shown between 1980 and 1990. As with the NBN dataset, the 5km grouping showed the smallest rise, with a rise from 12.30% in 1980 to 47.95% in 1990. After this, records continued to rise but at a slower rate than that of the NBN results. The highest value shown was for the 20km buffer result in 2010, which was close to 98% of new records falling within 20km of previous decadal results.

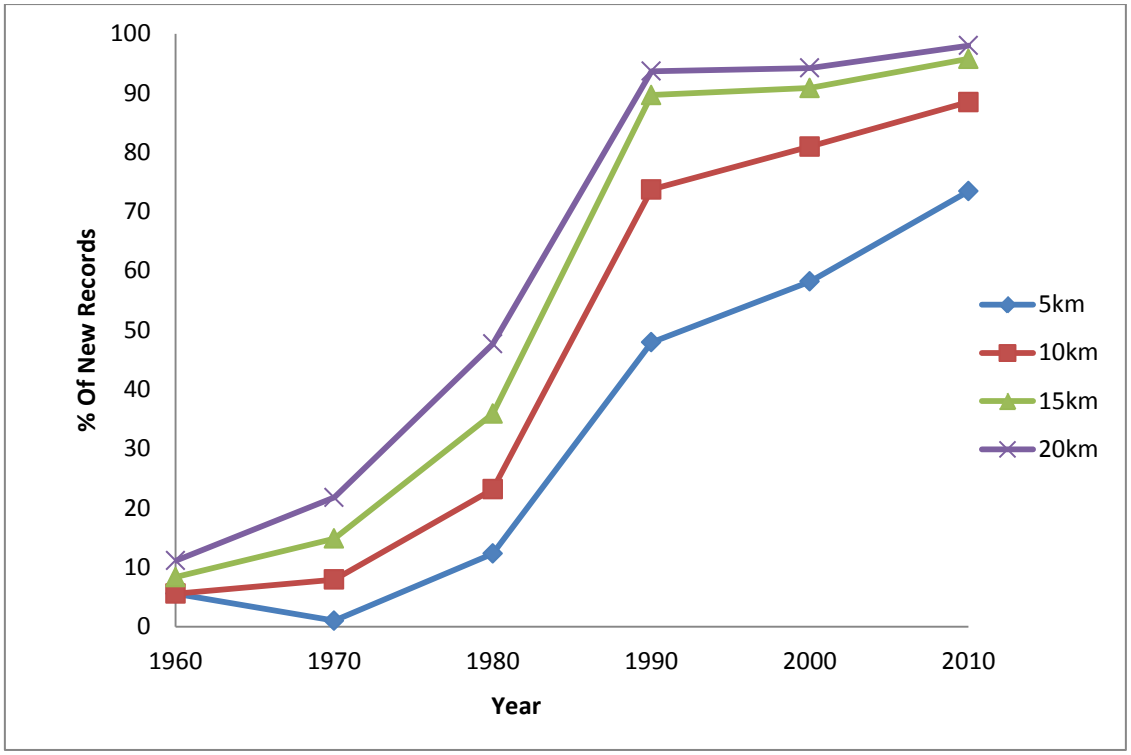

Figure 2.14. Percentage of new cases of *Crassula helmsii* falling within buffer zones of previous years records (BSBI Data). The four buffer zones are 5, 10, 15 and 20km and are shown independently.

Designated Sites Using UK Data

The Friedmann repeated measures test that was carried out for both year and sites variables returned P values of <0.05 for both NBN and BSBI datasets. Therefore, it can be concluded that the datasets are significantly different from the null hypothesis of no significant relationships existing between years or sites. On examining the graphs for evidence of this, it can be seen that some results lie outside the observed pattern. For the NBN data (Fig. 2.15), the NNR+2 and LNR data shows a distinct deviation from the other data. The BSBI dataset comparison (Fig. 2.16) the LNR and NNR data sets deviate from the main data grouping. These deviations indicate an increase in frequency of *C. helmsii* records per unit area when compared to other designated sites.

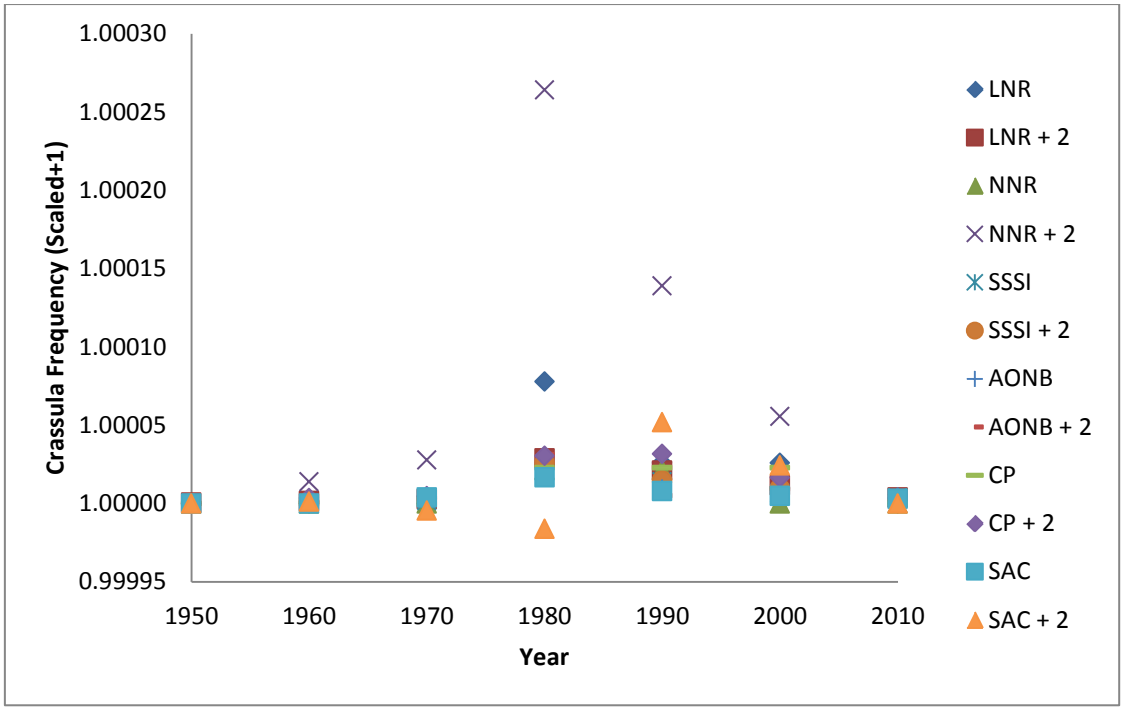

Figure 2.15. Comparison of *Crassula helmsii* frequency against decadal recordings for designated sites and their 2 km boundary buffer zones using the NBN database. Six different designated sites are shown, with their accompanying buffers, totaling 12 datasets.

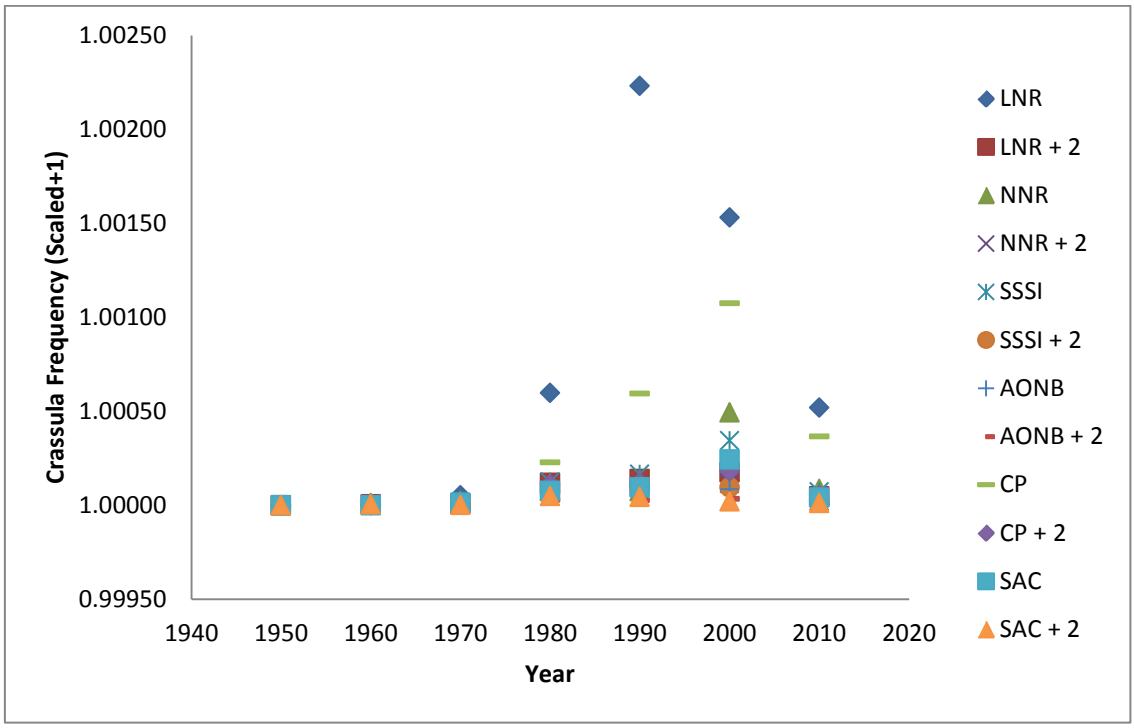

Figure 2.16. Comparison of *Crassula helmsii* frequency against decadal recordings for designated sites and their 2 km boundary buffer zones using the BSBI database. Six different designated sites are shown, with their accompanying buffers, totaling 12 datasets.

County Level Analysis Using Kent Data

Table 2.1. Regression comparison output of the county level analysis. The year of first recording was used as the dependent variable and distances to geographic features as the independent variables. The 'lake' and 'settlement' variables were both found to be significant to P=0.05.

Variables	P
Constant	0.001
A-Road	0.548
B-Road	0.176
Minor Road	0.957
Motorway	0.052
Coast	0.272
Railway	0.183
Lake	0.007
River	0.639
Settlement	0.008

Due to the large volume of data returned by the distance analysis using MapInfo, the Kent data set was analysed using a multiple regression test (Tab 2.1). The lakes data and settlements data, returned P values of <0.05. All other geographic features that were analysed in this way did not show a significant relationship to the data points.

Discussion

Records Overview Using UK Data

The overview of the data shows various trends depending on which of the date parameters are considered. The cumulative data for both data sets shows an increase in records of *C. helmsii*. When examining the decadal grouping, the number of records appears to be decreasing dramatically. These data manipulations therefore provide an example of how data display and subsequent interpretation is reliant on how the data is grouped. It is, therefore, unclear as to what the current situation of *C. helmsii* spread in the UK is. Distinguishing the slower

establishment phase of an invasion from the minimal sampling effort that new novel species afford is difficult (Aikio *et al*., 2010). It may be that both occur simultaneously, and so an obscured view exists of what the actual situation is during the first years of invasive establishment. Patterns of spread of invasives have been shown to slow and then inevitably stop increasing (Shigesda, 1994). A slowing effect is also shown to occur, when less suitable habitat is available for colonisation (Marco *et al*., 2011). Whether this is the case for *C. helmsii* is difficult to tell, as yearly records are showing a continual rise in the number of reported cases, whilst the longer term predictions are showing a decrease.

The peaks in data recording may be linked to data banding, with unknown data being labelled as collected in 1990 or 2000, where no exact year date is available. Some evidence for this would seem to be apparent on the yearly data pattern, with peaks at the start of new decades. It may also be due to an enhanced sampling effort in these years. It is uncertain whether this is due to data grouping or an enhanced effort in sampling and recording in those years. Whilst not disrupting the overall number of records, these artificial data groupings would be responsible for the peaks in the larger time banded data of the 5 yearly and 10 yearly increments.

Distribution Method Using UK Data

The investigation into dispersal distance for *C. helmsii* for both NBN and BSBI datasets showed very similar results. Until the 1980's, the majority of new records were found greater than 20 km from the previous decade's records. This would appear to indicate that the plant was spreading through long distance dispersal (LDD), at the outset of its naturalised discovery in 1956. During the 1980's the data indicates more short distance dispersal (SDD). The NBN dataset shows a peak value of 74.36% of new records within 20 km of a previous record in 2010. Though not as large a change, the 5 km buffer also shows an increase from 1.71% in 1980 to 13.47% in 1990. The BSBI data set also shows this sudden change between the 1980's and 1990's, with values close to doubling in all buffer sizes.

The change from LDD to SDD would seem to indicate a change that occurred once the population was sufficiently established. It is known that the plant was originally introduced as a horticultural plant for oxygenating ponds, and therefore was likely to be distributed by humans as an ornamental species. Propagule delivery in this manner has been shown to occur frequently (Higgins *et al.,* 2003). This may explain why LDD is shown as the main method of

spread before the 1980's. During the next 10 years until the 1990's, a greater level of SDD began, possibly due to the plant interacting with more natural means of dispersal, which has been shown to occur for other species (Carlquist, 1967; Figuerola and Green, 2002). The NBN dataset shows a decrease in SDD for the 5 km buffer, whilst the BSBI dataset show continual growth of the 5 km buffer towards SDD. Being that the BSBI dataset is larger than that of the NBN dataset, it is likely to be more accurate. It therefore indicates that species that spread by LDD (horticulture trade) and then change to favour SDD (natural as well as accidental human vectors) may require models that are distinct from 'natural' scenarios. Examples of both SDD and LDD dispersal occurring together for a species have been shown in previous studies (Gilbert *et al*., 2004; Giloli, 2013). There is however very limited research that looks at a combination of anthropogenic and natural spread.

The sudden change in the 1990's is unlikely to be due to a change in human behaviour. Though the plant began to gain more attention from a research perspective, wider scale public interest and awareness of *C. helmsii* did not start until the late 2000's. Therefore, this change towards a greater level of SDD must be attributed to another means, such as the idea of niche saturation. A study that looked at internet commerce as a method of distributing non-native macrophytes has shown that it was responsible for promoting LDD of species (Lenda *et al*., 2014). The increase in SDD shown by *C. helmsii* whilst internet sales of the plant are likely to have increased would be expected to show increases in LDD. This therefore supports the idea that by the time SDD became more pronounced, it had already spread across the country. *C. helmsii* was added to section 14ZA of the Wildlife and Countryside Act 1981 (Prohibition of sale of invasive non-native plants) in 2014 (Great Britain Wildlife and Countryside Act 1981/ Order 2014), so was not legally banned from sale until after the data was analysed for this study. Therefore at the point of data collection it is likely that it was still being spread by the horticultural trade.

Designated Sites Using UK Data

The use of the 2 separate datasets for the designated sites comparison returned different results (Fig. 2.15 and 2.16), with the NBN data highlighting LNR and NNR+2 and BSBI highlighting LNR and CP. This illustrates how the results of this analysis are dependent on which of the data sets are used, and the importance of utilising as many records as possible.

An explanation for the increase in records of *C. helmsii* per unit area for these three land designations is difficult to ascertain. An increase outside of a designation, as is illustrated by the 2 km buffer, would seem to suggest successful control has occurred within the designation. This is unlikely, as no control has been shown to be particularly successful (see Chapter 1). Mechanical control has been shown to increase fragmentation of *C. helmsii* (Hussner, 2009), so whilst it may have small scale benefits within a reserve, the side-effects could result in increased propagules delivery outside of the boundary of the site. This may have occurred in National Nature Reserves and be the reason for the increases within the 2 km buffer zones. National Nature Reserves fall under the management and control of Natural England, and therefore are likely to have more funding than other land designations. In the 2011/12 financial year, spending on by Natural England on NNR was £6.4 million, with an average spend per NNR of £44,500 (Natural England, 2013). Of this, more than one third (£2.2 million) was spent on contractors, which includes management of non-native species. As funds were available, and knowledge of *C. helmsii* fragmentation and enhanced spread is likely to have been lacking, this may show how management could have led to propagule release and subsequent species spread.

Country parks are often run and managed by county councils, whose priorities may not be devoted towards invasive species control but are weighted more heavily towards recreation (Lambert, 2006). This may be due to funding restrictions, lack of knowledge and identification skills and a general lack of concern for non-native species establishment. There are also often well visited by the public, with Kent having annual visitor numbers of 1.4 million people to its country parks (Kent County Council, 2014). As such, it has a large supply of anthropogenic vectors for propagules and subsequent species establishment. Dogs are also often allowed onto these sites, which can provide an even more efficient method of vector transport than humans, due to their habit of entering into water bodies.

Local nature reserves are managed by local authorities, and as such are likely to rely on volunteers and keen amateurs for species recording (Barker and Box, 1998). The economic pressures on local nature reserves means that carrying out detailed wildlife surveys are unlikely to be given much attention by local authorities (Kirby, 1993). Therefore, the records that are submitted from these designations are likely to be from the volunteer sector. Whilst this could result in erroneous recordings, it is likely that those amateurs that are keen enough to go out in the field to study wildlife are likely to have a supporting level of knowledge and ability. A result of this is that Local Nature Reserves are potentially well studied sites, as they

are often also used for educational purposes (Barker and Box, 1998). This could lead to an increased surveying effort on these sites, and as such greater numbers of records of *C. helmsii*. Control attempts may be less well informed due to this combined effort of local authority and volunteer group site management. This could result in fragmentation and greater dispersal of propagules, which in turn may be recognised by the greater surveying effort.

Fig. 2.7 shows a map of Kent with SSSI and buffer zones indicated. As many of the SSSI are at the coastline in Kent, the buffer zones would extend out into the sea, where *C. helmsii* is never found due to tidal movement and high salinity values preventing growth. There would therefore be a bias towards the buffer zones, as they would factor in a lot of area that *C. helmsii* could not be found in. A correction for this could be applied, but would require manipulation of the mapping layers. Attempts have been made to use this form of buffer analysis in epidemiological studies, with interference from outside variables causing difficulty (East *et al*., 2008).

County Level Analysis Using Kent Data

Significant probability values for lakes and settlements were returned by the regression analysis of the Kent data set. The lakes significance may be explained by *C. helmsii* being an aquatic macrophyte that favours still waters. A relationship only with lakes may also explain why the rivers analysis did not return a significant value. The settlements significance may be due to *C. helmsii's* initial use in the horticultural trade, and as such inclusion within garden assemblages.

Correlation to settlements and therefore likely initial human vector supports the findings of the spread patterns being initially via LDD, as the combination of garden ponds and mail-order plants (as was the initial method of distributing *C. helmsii*) is supported (Lenda *et al*., 2014). The movement from garden ponds to natural water bodies may have been via natural vectors. There is also a possibility that, due to the uncontrollable nature of the plant, disposal into natural areas by gardeners could have occurred through the movement of vegetative waste from ponds, thus inoculating native waters. Pre-meditated dispersal is also a possibility, whereby well-meaning people may be intentionally populating native water bodies with the plant in order to 'increase' biodiversity.

Other invasive species that can be initially sourced from the horticultural trade could be investigated using these mapping systems, to investigate whether this trend can be found for

other species. This would support other studies that have shown non-native species that have escaped from gardens to be a significant source of the non-natives present in Britain (Dehnen-Schmutz *et al*., 2007). It may allow for more targeted predictions of dispersal to be calculated, thereby allowing for more resources to be deployed in such a way as to be most effective. Population density was found to act as a predictor for *Cameraria ohridella* (Horse Chestnut Leafminer) in a study of dispersal patterns (Gilbert *et al*., 2004).

Limitations

Whilst the records gained from both the NBN and BSBI are likely to be a good representation of the spread of *C. helmsii*, it is unlikely that they are fully representative of all growing locations. Databases have developed over time to be easier to submit sightings to, with the use of e-mail and online forms. This ease of delivering sightings is likely to have enhanced the number of records, compared to when more time and effort was required. The level of completeness of these records should therefore be considered.

The accuracy of the records may also cause discrepancies in the results, as they are often only to hectad level (10kmx10km). When the buffers are applied, this would cause a compounded error, which may lead to the overlapping of results from the subsequent decadal band not falling within the target area, and therefore causing inaccuracy. This is especially important for the county level data, as direct measurement to geographical features were used. Though the county level records were more accurate as they were site specific, some inaccuracy is still likely, which would again be compounded by the analysis.

As the analysis was geographically limited to just the UK, the number of locations that *C. helmsii* can disperse to is limited by geographic boundaries. As the number of records increases, the probability of records becoming more localised to each other also increases. Therefore, a transition from LDD to SDD may be returned as the number of records increases over time. For example, a new record may be due to a LDD event from the south of England to the north of England (such as a member of the public buying the plant for their pond). A SDD event may then be responsible for moving a propagule from this specimen into a neighbouring wildlife reserve, which is recorded by the land manager. If *C. helmsii* was already present on the reserve, but was not spreading by SDD, the analysis methods in this study would assume that movement had occurred from within the wildlife reserve (therefore under representing a LDD event). In order to remove this as an error, knowledge of all locations of *C. helmsii* would

be required, which includes both private and public spaces. This would be difficult to achieve without a large surveying effort, and likely only be realistic in a small area due to limited participation.

Conclusion

The use of GIS software and large datasets has enabled an investigation into a range of attributes of the distribution method and spread of *C. helmsii*. By using two separate datasets, it has been shown how differences can occur which could lead to poor decisions being made in the management of invasive species. It has also been shown how, depending on which model is used and time increment analysed, the projection of invasive species and forecasting models can draw different conclusions.

By studying designated sites, a range of factors have been addressed which include management practices, site description and overall site objectives. These have profound effects on species diversity and could be used as a way of studying different control techniques, subsequent dispersal dynamics and follow up establishment on a species after a control has placed pressure on a species to disperse.

The use of smaller datasets of a more specific area with a large volume of geographic data has enabled the correlation of species distribution to identify any habitat preference or vector pathways that may be present.

The use of data and GIS analysis is a useful tool in studying not only previous records, but that of future patterns and movement of a species. By using these methods with a number of species that could include but not be limited to other aquatic non-natives, a useful tool in measuring species spread could be developed.

In the following chapters, the main research questions on the ecological impact of *C. helmsii* upon native species will be addressed. Chapter 3 describes the arrangement of the main study, concentrating on the field work requirements that were integral to collecting the data set for comparison in subsequent chapters. The sites identified within Kent and East Sussex that were used in this chapter were beneficial when looking for suitable study sites for field work. However, as will be addressed in the next chapter, limitations due to access permissions and safety concerns prevented all of the sites identified within this chapter from being studied.

Chapter 3 – Surveying Strategy and Methodology

Introduction

Studies that examine the impact of *C. helmsii* on native species are lacking from the literature. Some studies have been carried out, but are minimal in both size and detail. Evidence of the suppression of an *Elodea* spp. in an artificially managed lake was the first to suggest an environmental impact (Dawson and Warman, 1987). Anecdotal suggestions of suppression exist, which include the suppression of *Ludwigia palustris* (Water Purslane) and *Galium debile* (Slender Marsh Bedstraw) in a pond system within the New Forest, Hampshire (Byefield, as stated in Dawson and Warman, 1987). A study of newt conservation found only delayed breeding maturity in *Lissotriton vulgaris* (Smooth Newts), but no overall loss of reproductive success (Langdon, 2004). Within this study, examination of macrophyte assemblages and seed banks found no significant difference between invaded and control sites. Hussner (2009) studied *C. helmsii* in lake systems, and found displacement of native species. This was, however, linked to nutrient enrichment and increased growth rather than direct competition. Due to the lack of data that investigated the impact of *C. helmsii* upon native species, a study that would investigate this was constructed.

In order to carry out a comparative study between invaded and uninvaded communities, sites were identified where *C. helmsii* was present, as well as those where it was not. To reduce variability and create a viable comparison, the uninvaded control groups were located as close to the invaded groups as possible. Approximately 100 sites in total were sought, with an approximate split of 80 with *C. helmsii* and 20 without. The Botanical Society for the British Isles (BSBI) provided a dataset of known locations of *C. helmsii* (Lockton, 2012). This was cross referenced with data provided by Kent and Medway Biological Records (KMBRC, 2012).

Four categories of data were investigated, with subsequent samples collected from each survey location. These were:-

- Water chemistry (Both in-field and for laboratory analysis).
- Invertebrate diversity
- Plant diversity
- Seed banks (By soil coring and seed separation).

Location of sites

Access to sites managed by large conservation organisations were favoured, due to the likelihood of correspondence and permissions being gained. Whilst attempts were made to gain access to sites under private ownership, the ability to gain a channel of communication with the owners was difficult to establish. Therefore, emphasis was placed upon larger organisation, where contact details were available and public access was permitted. These included:

- Natural England (NE)
- Royal Society for the Protection of Birds (RSPB)
- Kent Wildlife Trust (KWT)
- Kent County Council - Country Parks Department
- Local Parish Councils (Eastry Parish Council)

From previous work on *C. helmsii*, access had already been agreed for two of the proposed sites. These were Blean Woods, under the control of the RSPB, and Stodmarsh National Nature Reserve, under the control of Natural England.

Timings

The surveys were carried out between May and August 2013. A summer survey was preferable, as it allowed for a greater diversity of plants and invertebrates to be present, and gave a more complete diversity ranking for the site (Howard, 2002). Identification at this time of the year is easier due to more morphological features being present and distinguishable (Haslam *et al*., 1982). Water levels were at their lowest for the time of year, which enabled the riparian margin to be accessed more easily.

Samples were collected in a single surveying season, rather than spreading the sample collection across multiple years. This reduced the possibility of annual variations, such as temperature and rainfall, causing large variations in species composition. The water chemistry was also more likely to remain stable and comparable in this manner, as winter temperature change and dilution factors, as well as changes in land management, have been shown to cause variability between years (Ohman *et al*., 2006; Sager, 2009; Muller and Tankèrè-Muller, 2012). Some sites were located close to farmland, and so by carrying out the sampling work in

this constricted time frame, it was hoped that any effects from surface run-off into the water bodies would be minimised.

Due to the nature of some sites being protected wetlands, important for breeding birds, survey permissions were felt unlikely to be given during the more vulnerable times of February to April. This correlated well with the previously stated factors for summer surveying.

Bio-Security

One of the major concerns when studying a non-native species in the field is the possibility of spreading it to new areas. Due to the ease of fragmentation of *C. helmsii*, this is a known problem and could lead to the development of new colonies. In order to decrease the risk of such, control sites were always sampled before *C. helmsii* sites, if both were being sampled on the same day. This ensured that there was a reduced chance of fragments being delivered to the control sites on equipment or footwear. Between surveys, the equipment was inspected to ensure that it was clean from propagules.

As a precaution, all equipment was washed and allowed to dry, as an additional measure to the visual checks. The use of a secondary net head was also adopted with a system of colour coding in place to ensure that no contamination occurred between *C. helmsii* sites and control sites.

Sampling Locations

By examining the list of records of known locations of *C. helmsii* growth, it was apparent that it grew across a wide range of habitats and waterbodies. These included coastal marshes, heathland bogs, woodland ponds, farmland ditches and reservoir grass margins (KMBRC, 2012). To ensure that these were represented, sites were selected that reflected these habitats. It was also deemed necessary to find control sites that were similar not just spatially, but also with the same physiochemical and environmental conditions as the invaded sites. These sites were spread across Kent, and into the eastern edge of East Sussex.

Access to sites and permissions for sampling were integral to the study being able to proceed. Due to this, records that fell on land that appeared to be under private ownership with no apparent methods of contacting the owner were disregarded. Emphasis was placed on larger

sites, with either public access available or where access could be arranged with the organisation that owned or managed the land. Contact with site managers commenced a year before sampling was planned to occur, with regular correspondence to ensure access remained available for surveying and sample collection. An inspection visit was carried out for most sites, to distinguish the number of sampling locations available, ease of access and to confirm the status of *C. helmsii* presence.

In total, 18 different locations were visited with a total of 82 individual sampling sites (Fig. 3.1) (More accurate GPS coordinates for each of the sampling locations can be found in the appendix). Not all measurements could be taken from each location due to access arrangement, disturbance issues and caveats placed on the surveying work by the landowners or land managers (Tab. 3.1).

Figure 3.1. An overview of the site locations where sampling occurred, indicated by green circles. GIS layers are of ordnance survey road layer and British Geological Bedrock layer. A range of sites were sought across the county in order to provide different biotic and abiotic conditions.

Table 3.1. The sample types and numbers from each site. Each site is divided into the four study areas, and whether *C. helmsii* was present or absent (control).

Site name	**Beacon Wood**		**Bewl Water**		**Blean Woods**		**Bough Beech**	
	C. helmsii	Control	*C. helmsii*	Control	*C. helmsii*	Control	*C. helmsii*	Control
Plant Surveys	2	0	5	0	2	0	5	1
Invert. Surveys	2	0	0	0	2	0	5	1
Water Surveys	2	0	5	0	3	0	8	1
Soil Surveys	2	0	5	0	2	0	5	1

Site name	**Dungeness**		**Eastry**		**Fowlmead**		**Hothfield**	
	C. helmsii	Control	*C. helmsii*	Control	*C. helmsii*	Control	*C. helmsii*	Control
Plant Surveys	5	1	2	0	0	3	2	4
Invert. Surveys	5	1	2	0	0	3	2	4
Water Surveys	5	1	2	0	0	3	2	4
Soil Surveys	4	1	2	0	0	3	2	4

Site name	**Oare Marshes**		**Oare Gunpowder**		**Orlestone Forest**		**Perry Woods**	
	C. helmsii	Control	*C. helmsii*	Control	*C. helmsii*	Control	*C. helmsii*	Control
Plant Surveys	5	2	0	3	0	2	2	0
Invert. Surveys	5	2	0	3	0	2	2	0
Water Surveys	5	2	0	3	0	2	2	0
Soil Surveys	5	2	0	3	0	2	2	0

Site name	**Romney Marsh**		**Rye Harbour**		**Rye Street**		**Sevenoaks Reserve**	
	C. helmsii	Control	*C. helmsii*	Control	*C. helmsii*	Control	*C. helmsii*	Control
Plant Surveys	2	0	5	0	5	2	5	1
Invert. Surveys	0	0	5	0	5	2	4	1
Water Surveys	0	2	5	0	5	2	5	1
Soil Surveys	2	0	5	0	5	2	5	1

Site name	**Shorne Woods**		**Stodmarsh**			Totals	
	C. helmsii	Control	*C. helmsii*	Control		*C. helmsii*	Control
Plant Surveys	5	0	5	2	**Plants**	57	21
Invert. Surveys	4	0	5	2	**Invertebrates**	48	21
Water Surveys	5	0	5	2	**Water**	59	23
Soil Surveys	5	0	5	2	**Soils**	56	21

Site Descriptions

Fowlmead Country Park

Fowlmead Country Park is located between the towns of Sandwich and Deal, on the east coast of Kent. Originally the site of the Betteshanger colliery, the site was redeveloped into the country park when SEEDA (South East England Development Agency) gained the lease in 2000 (Fowlmead Country Park, no date). The site has been designed for amenity and wildlife use, with a mix of cycle and walking tracks and with wildlife areas such as woodland, lakes and scrub. The lakes are where the *C. helmsii* records are recorded as being present, though not across all sites.

Initial surveys in the winter of 2012 found no evidence of *C. helmsii*. One of the lakes was identified as a suitable control site, with no records of *C. helmsii* and no visible presence of the plant.

Figure 3.2. The control lake at Fowlmead.

On the return visit on 2nd June 2013 no evidence of *C. helmsii* could be found on any of the waterbodies. It was therefore decided that this site would become a full control site, with the water chemistry variability being noted as a possible source of error due to the artificial geology. Three control sites were surveyed. Another two possible waterbodies that had been identified by analysis of records and aerial photography were found to be dried out and so were unavailable for surveying. No evidence of *C. helmsii* was found at any of the waterbodies studied, which was in disagreement with the records.

Figure 3.3. Clockwise: Ordnance survey site location, Google Earth site location and survey location C3 at Fowlmead Country Park.

Eastry Village Pond

Eastry is a village in East Kent, where records had shown that *C. helmsii* was present in the village pond, known as 'Buttsole Pond'. A management plan had been in place for the pond from 2008, and so active management could be seen to be occurring which targeted the *C. helmsii* (White Cliffs Countryside Project, 2008). Evidence of this was seen to be via physical removal and drying out on the riparian margins. Examination of the pond in 2012 had noted 2 distinct areas where surveys could be carried out, of approximately 10m length. Due to the pond being a single water body, no control was available.

On the return visit on 2nd June 2013 both sites were sampled that had previously been identified.

Figure 3.4. Clockwise: Ordnance survey site location, Google Earth site location and survey location 2 at Eastry.

Stodmarsh NNR

Stodmarsh is a National Nature Reserve that extends along the southern edge of the river Stour. It is an internationally important site, consisting of marsh, reed bed, riparian, wet woodland and open grassland habitats (Natural England, 2009). The site is also one of the few in the county to support *Segmentina nitida* (shining ram's-horn snail) within the ditch system (Killeen, 2000).

Stodmarsh had been used for preliminary work on *C. helmsii*. A map of areas of known presence was supplied by Natural England (Plunkett, 2011), and was supported by a site visit in 2011. Therefore preliminary surveys were not required for the summer 2013 survey visit.

The area studied was that of a former lawn farm, which was under reversion to natural grassland (Etherington, 2011). Grazing livestock (Konik Pony) were present on site, with water management by ditch and scrape systems. One area had recently undergone large scale mechanical clearance management in January 2012, due to the establishment of *C. helmsii*.

Due to the sensitivity of the site for birds, access was arranged for surveys to begin as early as possible to prevent undue distress. On the main sampling visit on 5th June 2013 seven separate sites were identified, which included five *C. helmsii* sites and 2 control sites.

Figure 3.5. Clockwise: Ordnance survey site location, Google Earth site location and survey location 5 at Stodmarsh.

Blean Woods NNR

Blean Woods is a large complex of woodland that surrounds the city of Canterbury, Kent. Previous work on *C. helmsii* had been conducted on the site, and so it was decided to include the pond systems in the main survey. The woodland is managed by a range of landowners and conservation bodies, with the area of study concerned being managed by the RSPB. Two pond systems were known of, and so access was sought from the RSPB for further study work in 2013.

Due to the protected nature of the site, the RSPB had requested that specific research criteria be completed and submitted to their ecology group. This resulted in access permissions being granted for the summer, 2013.

The pond systems are fed by the Sarre Penn stream, with both ponds acting as drainage areas (Explore Kent, 2005). This meant that, although joined by this narrow feature, the waterbodies were lentic where *C. helmsii* was present. A preliminary study of the 2 ponds had discovered easy access to one, with navigation around the entire pond margin possible. The secondary pond was found to be more vegetated, with no clear riparian margin and access through the woodland difficult. Management of the first pond system could be seen in the form of mechanical removal, with spoil containing growing specimens of *C. helmsii* present in the neighbouring woodland understorey (which remained viable and continued to grow due to the clay, moisture rich soils).

The accessible pond was surveyed in two locations from its south west and north east corners. The more secluded pond was located, but due to health and safety issues of steep sided and poorly defined banks, it was not accessible for a full survey. A water sample was however collected.

Figure 3.6. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Blean.

Perry Woods Local Nature Reserve

Perry Woods is a small woodland complex near the village of Selling, between Canterbury and Faversham. The geology of the site is unusual for the area, being an uprising of gravels and sands on the edge of the chalk North Downs (Perry Woods, 2014). Due to this accumulation of sand, the site is free draining and acidic, therefore supporting conifers and rhododendron. At the southern edge of the woodland is a large pond network. This pond network was noted as

showing presence of *C. helmsii*, and so was inspected for the species. Its presence was confirmed in the winter of 2012.

Due to the size of the pond, 2 areas were identified for sampling. The management of the site was unclear, with no visible activity being seen. The surveys at Perry Woods were carried out on 20th June 2013, with two *C. helmsii* surveys being completed at the site.

Figure 3.7. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Perry Woods.

Hothfield Heathlands SSSI

Hothfield Heathlands (formerly Hothfield Common) is the largest remaining remnant of heathland and acid bog system in Kent (Natural England, 2013). It is located to the north of

Ashford, and owned by Ashford Borough Council. The management of the site is carried out by Kent Wildlife Trust, through a mixture of felling, spraying and grazing.

Only 1 of the bog systems, at the northern end of the reserve, had records for the presence of *C. helmsii*. Inspection of the site in the summer of 2012 confirmed this, with the plant being present across a wide area of the bog. Control practices were in place, in the form of shading by black plastic. Additional surveys were agreed at a site nearby to provide control samples, on a reserve called 'New Fen' (Rickards, 2013). Access permission was granted by Ian Rickards, site warden for Kent Wildlife Trust, with the surveys being carried out on 11th June 2013. Four control sites and 2 *C. helmsii* sites were surveyed.

Figure 3.8. Clockwise: Ordnance survey site location, Google Earth site location and survey location C3 at Hothfield Heathlands.

Shorne Woods Country Park

Shorne Woods is a country park owned and managed by Kent County Council. It is located between Gravesend and Rochester, in the north west of Kent. It is a site that combines management for both amenity and wildlife value, with an emphasis on education and use by schools (Kent County Council, date unknown). The presence of *C. helmsii* was found by using records, with a follow up visit in the August 2012. All waterbodies on the site showed presence of *C. helmsii*.

Surveys were carried out on 17th July 2013, with five *C. helmsii* sites surveyed. No control sites were available due to the growth of *C. helmsii* across all waterbodies.

Figure 3.9. Clockwise: Ordnance survey site location, Google Earth site location and survey location 4 at Shorne Woods Country Park.

Oare Gunpowder Works CP

Oare Gunpowder Works is located to the west of Faversham, near to the village of Oare. It is a Country Park owned by Swale Borough Council and managed by a range of conservation bodies (Oare Gunpowder Works, date unknown). It is an important historical site due to the remains of a gunpowder mill that was operating up until the early 20th century. It is currently managed for a combination of amenity and wildlife aspects, whilst retaining the stewardship of the remains of the building and infrastructure.

No records for *C. helmsii* were found in the literature, and a preliminary inspection of the site found no evidence of the plant in any of the lakes, pools or ditches. It was therefore decided that this site would provide control groups for the study. Access permission was not deemed necessary due to the site being open to the public. The site was visited on 6th June 2013, and three control surveys were conducted.

Figure 3.10. Clockwise: Ordnance survey site location, Google Earth site location and survey location C2 at Oare Gunpowder Works.

Oare Marshes

Oare Marshes is located on the north Kent coast, between the mainland and The Swale, a narrow waterway that separates the Isle of Sheppey from the mainland. It is owned and managed by Kent Wildlife Trust, primarily due to its diverse water bird numbers (Kent Wildlife Trust, 2014). The habitats are a mix of wetland and marshland, with artificial scrapes and floods. Though coastal, the site is isolated from the main Swale channel, and therefore the salinity and subsequent biota of the site are distinct from that of the coastal marshes that lie over the sea wall. The site is predominantly managed by winter grazing, with the use of Konik pony and Highland cattle to reduce scrub growth and keep the grassland sward low and diverse (Duvall, 2013).

Presence of *C. helmsii* was found in one of the ditch systems. Sampling permission was gained from Kevin Duvall, site warden for Kent Wildlife Trust, with the main sampling effort occurring on 29th May 2013. On the return sampling visit, two control sites were located and sampled prior to five *C. helmsii* surveys.

Figure 3.11. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Oare Marshes.

Rye Street – RSPB Reserve

The RSPB reserve at Rye Street is situated on the Isle of Grain, and makes up part of the north Kent marshes complex. The Rye Street reserve itself is to the west of Cliffe, and the east of Northward Hill reserves. These are two large RSPB sites, with the adjoining Rye Street reserve acting as a link between the two. The habitat of Rye Street is predominantly grazing marsh, with sheep grazing as management in 2013. The site is a network of fields and ditches, with the ditches acting as channels across the site for drainage. Within these ditches was where *C. helmsii* was located.

The main surveys took place on 16th June 2013. Two control sites were identified and sampled initially, with a further five sites where *C. helmsii* was present being surveyed afterwards.

Figure 3.12. Clockwise: Ordnance survey site location, Google Earth site location and survey location 2 at Rye Street.

Bough Beech Reserve

Bough Beech reserve is located in west Kent, at the north of the Bough Beech reservoir. Kent Wildlife Trust manages the site, in conjunction with South East Water who operates the reservoir (Basset, 2013). The reserve is made up of grassland, scrapes and a small river which acts as a feed for the reservoir. It is noted for its diverse bird populations and is managed accordingly. An initial visit was carried out to identify areas for survey in the spring of 2013. *C. helmsii* was present across the site, and so a range of different habitats and areas were available for sampling. A separate wildlife pond that was not connected to the main water flow and free from *C. helmsii* growth was designated as a control site. Due to the management of the site and the need to hold water during the winter due to instruction by South East Water, the water levels were high. This meant that actual survey locations were not attainable due to

the change in site characteristics in the summer. Previous management of *C. helmsii* was by herbicide spraying.

The surveys were carried out on 31st July 2013. One control site (the wildlife pond) was surveyed, with five *C. helmsii* surveys also being conducted. An additional three sites were surveyed beyond the main reserve, where the plant had colonised recent scrapes that had been installed for wildlife enrichment. Only water samples were collected for these three locations.

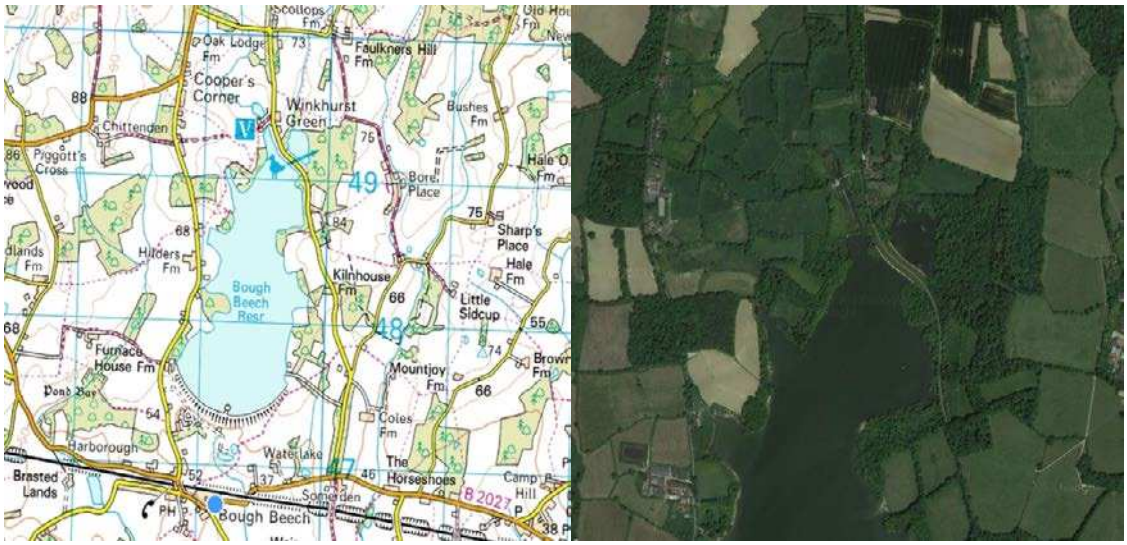

Figure 3.13. Clockwise: Ordnance survey site location, Google Earth site location and survey location 2 at Bough Beech.

Sevenoaks Reserve

The Sevenoaks reserve is managed by Kent Wildlife Trust and is the site of a former gravel works. It is composed predominantly of large lake systems with adjoining pools and waterside vegetation. Records suggested that *C. helmsii* was present in a range of locations, and on contact with the site warden this was confirmed (Clerici, 2012). A preliminary visit in the spring of 2013 identified areas of *C. helmsii*. Breeding bird disturbance was noted as a possibility for this site, and so surveys were proposed for late summer to avoid nest disturbance.

Sampling took place on 29th July 2013, with 5 *C. helmsii* locations and 1 control site surveyed.

Figure 3.14. Clockwise: Ordnance survey site location, Google Earth site location and survey location 5 at Sevenoaks.

Beacon Wood Country Park

Beacon Wood Country Park is located near to the village of Bean, in the north west of Kent. The site has a diverse history, being used for fruit production, timber supply, gunpowder manufacture and most recently clay extraction (KCC, date unknown). The site is a mixture of deciduous woodland and shallow ponds, with management by Bean parish council for amenity and wildlife enhancement.

Sampling took place on 21st July 2013. Due to the low water levels only one area of water was found, though due to its large size enabled two separate sampling locations to be found. No control sites were available.

Figure 3.15. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Beacon Woods.

Romney Marsh Reserve

The Romney Marsh reserve is located on the south coast of Kent, next to the town of Romney Marsh. The reserve is comprised of two distinct areas. The first is managed by Kent Wildlife Trust and the second is located on the neighbouring land and is managed by the Romney Marsh Countryside Partnership.

Sampling took place on 8th August, 2013. It was discovered that the Kent Wildlife trust part of the reserve no longer showed presence of *C. helmsii*, due to successful management (the original pond had been completely re-laid, with new plant species being introduced artificially). It was therefore decided that it did not qualify as a native pond, but water samples were taken as controls.

On the neighbouring reserve managed by the RMCP, *C. helmsii* was found. Due to the low water level, water was not present and so water and invertebrate sampling could not occur. Two plant diversity and soil cores were taken.

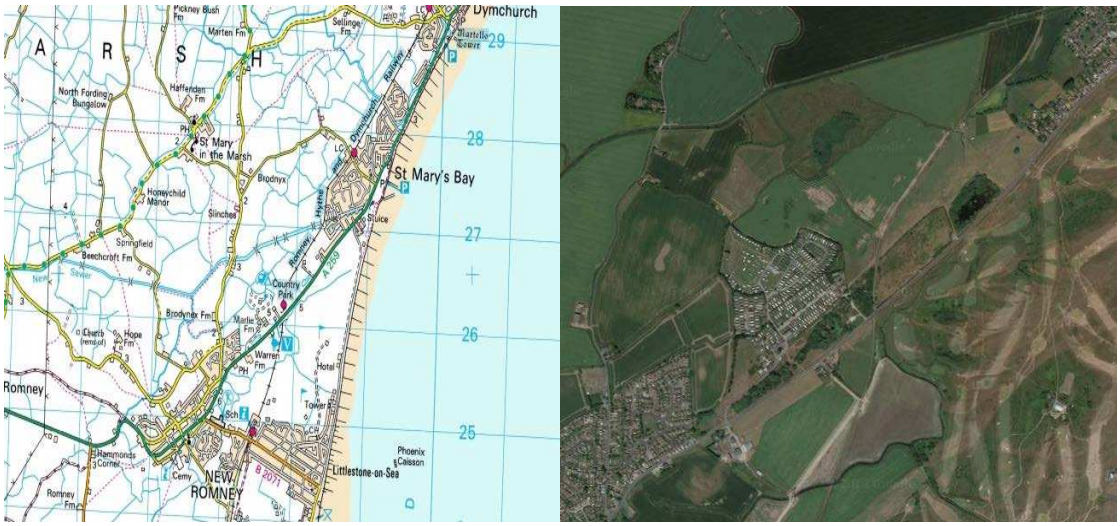

Figure 3.16. Clockwise: Ordnance survey site location, Google Earth site location and survey location 2 at Romney Marsh.

Dungeness RSPB Reserve

The reserve at Dungeness is located on the southern coast of Kent, near to the border with East Sussex. The site is managed by the RSPB for wildlife diversity. It is a popular site for birds, due to its lakes, ponds, shingle, exposed sands and neighbouring fields. Its location next to the English Channel makes it a popular site for observing migration patterns to and from the continent (RSPB, 2014). Management techniques are diverse, ranging from habitat creation, scrape establishment and grazing regimes.

Due to the high level of protection accorded to the site, access permission was required through the RSPB's ecology department. Due to known presence of Red Data Book species of freshwater invertebrates, sampling and identification was only allowed if carried out in the field and on site. A previous sampling effort had found that 43% of identified invertebrates were RDB categorised (Booth, 2012). As this was felt not achievable, and so as to ensure access was permitted for the other sampling types, invertebrate samples were not collected.

The main sampling effort took place on 22nd August 2013. Due to the underlying geology of one sampling location, a soil core could not be taken as a topsoil layer was too thin. The sampling locations included ditches and ponds, on sands, gravel and silt. In total, one control location and five *C. helmsii* sites were sampled.

Figure 3.17. Clockwise: Ordnance survey site location, Google Earth site location and survey location 4 at Dungeness.

Rye Harbour SSSI

Rye Harbour is located to the south of the town of Rye, on the Kent and East Sussex border. The site was identified as having *C. helmsii* present from the records, which was confirmed on consultation with the site warden (Yates, 2012). Rye Harbour reserve is a mixture of grazing marsh and pond and ditch systems. It also supports shingle uprising and exposures due to its coastal nature. Due to its coastal nature, the salinity is variable across the site, with subsequent species associations resulting (Wild Rye, date unknown). It is managed for wildlife in conjunction with strong access and educational attributes. The site is managed by Sussex Wildlife Trust in cooperation with a local community conservation group (Yates, 2013).

Access permission for sampling was granted in 2012. The main sampling took place on 13th August 2013. Five *C. helmsii* samples were taken, with no appropriate controls being found.

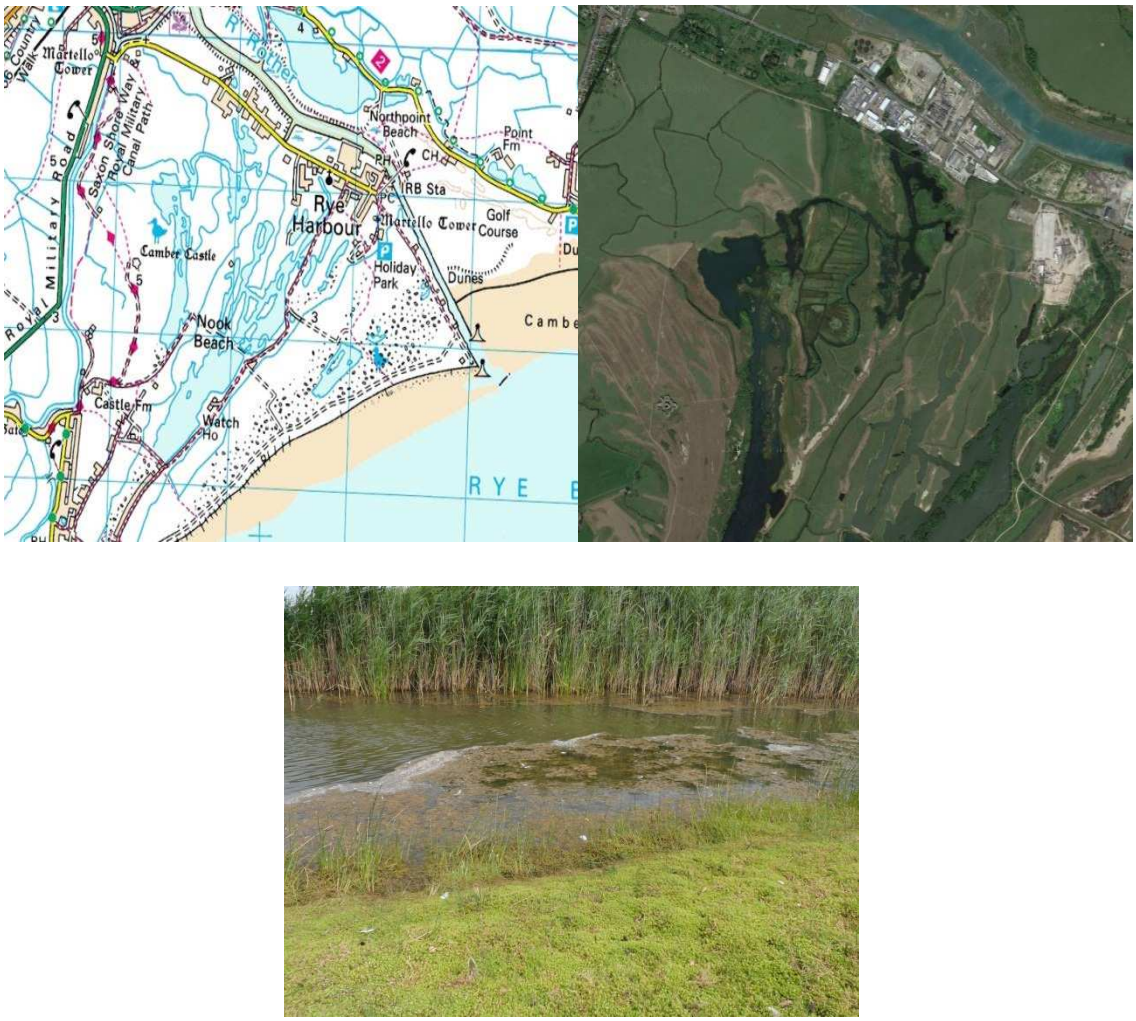

Figure 3.18. Clockwise: Ordnance survey site location, Google Earth site location and survey location 2 at Rye Harbour.

Orlestone Forest

Orlestone Forest is located to the south of Ashford Kent. It is a large woodland complex, with a range of stakeholders including the Forestry Commission, Kent Wildlife Trust and private landowners. The woodland surveyed is more specifically known as 'Burnt Oak Wood', which contains several ponds. The site is not open to the public and contains no public footpaths. From previous work it was known that these ponds existed, but no records of *C. helmsii* for the site could be found. A visual inspection of the ponds in 2012 confirmed this, and so the two ponds were deemed suitable control locations for sampling.

Sampling occurred on 22nd August 2013. Two individual control sites were sampled within the woodland complex, with no *C. helmsii* found.

Figure 3.19. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Orlestone Forest.

Bewl Water

Bewl Water is a large reservoir in west Kent, near to the village of Ticehurst. It is managed by South East Water. The site is also used for recreation activities. The records indicated that *C. helmsii* was present on the eastern edge of the site, extending along the margins of the drawdown of the water body. Limitations were placed on the sampling effort, with invertebrate sweeps not available.

The site was surveyed on 26th July 2013. Four samples were taken from the northern edge of the eastern arm of the reservoir, with one sample being taken from the southern side. No appropriate locations were found for control sampling.

Figure 3.20. Clockwise: Ordnance survey site location, Google Earth site location and survey location 1 at Bewl Water.

Sample Analysis

Plant sampling

For each sampling location, plants were identified along a 10 m section of the riparian margin of the waterbody, which was walked 3 times to record the plant species present. The survey area included growth up to the winter high water line, which was visible during summer

surveying due to sudden species composition change or a band of dead plant material. Aquatic plants that were within 3 m of the bankside were also recorded. Plants that could not be immediately identified or keyed out in the field were photographed for later identification.

The study initially followed the methods set out by the Predictive System for Multimetrics (PSYM) (Howard, 2002). This method requires a complete plant list for each survey location to be created, which is used to generate rarity scoring. It was found however, that the individual plant scores for PSYM were outdated (Williams, 2013). It was also found that the system could only be used in full for permanent ponds, and no other lentic or temporary waterbodies (Williams, 2013; Van den Broeck, 2015). A lack of a clearly defined method for analysing macrophyte assemblage in lentic waterbodies has been identified (Van den Broeck, 2015), with modifications and adaptations often required for these under-studied ecosystems.

It was, therefore, decided to incorporate the PSYM metrics, but also use additional scoring systems. The use of plant diversity and subsequent scoring systems have been used in many previous studies that have evaluated botanical site composition (Linton and Goulder, 2000; Williams *et al*., 2003; Nicolet *et al*., 2004; Smith *et al*., 2006; Cereghino *et al*., 200; Gerber *et al*., 2008). Online databases are available that give empirical values for distribution, which can be used for generating rarity scores (NBN, 2013, BSBI, 2013). These also included regional scores, generated from Kent distribution maps (Philp, 1982; 2010). Latimer (2009) showed how national scoring systems can lack the ability to show impact sensitivity, and so more local scoring metrics were felt to be important. Chapter four gives a more detailed commentary of the use of plant scoring metrics in this manner.

Plant species lists were also analysed using MAVIS software. MAVIS – Modular Analysis of Vegetation Information Systems was developed by the Centre for Ecology and Hydrology (Smart, date unknown.) MAVIS software allows for the analysis of site characteristics using plant diversity and/or abundance. The software was developed from information gained from the Countryside Survey 2000 and the Countryside Vegetation System. For this study, the software provided results for light, pH, fertility and wetness, which were then used for further analysis. Chapter four gives a more detailed analysis and the results of the plant study.

Invertebrate Sampling

Macroinvertebrates were collected from each sampling location using a 3 minute pond net sweep using a Wolf pond net of dimensions 30 cm x 35 cm. Two separate net heads were

used, each being designated to either a control or *C. helmsii* site by the use of coloured tape for identification. A single, 1.8 m pole was used, with net heads having an interchangeable mechanism.

The 3 minute sampling time was carried out in accordance with the PSYM methodology, developed by The Pond Conservation Trust. Each sub-habitat was designated a set amount of time within this 3 minutes, to allow for a stratified sampling attempt. (For example, a margin of 10m showing 2 types of habitat across 2 x 5 m would be sampled for 1.5 minutes each) (Pond Conservation Trust, 2002).

The resultant sample was decanted into a plastic container. Any visible amphibians and large vegetative matter were removed. A small volume of water from the swept waterbody was included in the container. For storage, the samples were refrigerated for 24-48 hours after collection. Samples were placed into approximately 50 ml of formalin (10%), buffered with sodium tetraborohydrate (borax). This enabled fixing of the specimens, which were kept in formalin for 24-48 hours. This solution was washed off, with samples being transferred to 90% IMS for long term storage.

Identification of species was carried out approximately 4 months after storage. Specimens were individually identified using a table mounted lens and digital microscope. This allowed for photographs to be taken easily, for cross referencing to aid with identification. Species were keyed out to family level (Quigley, 1977; Croft, 1986; Smith, 1986). A single specimen for each family was sought to allow for the creation of a diversity list for each sampling location. These lists were scored using BMWP scores as well as a metric devised just for the samples collected. Van den Broeck *et al*. (2015) discusses how methods of evaluating small (<50 ha) and/or temporary water bodies are still lacking from the literature, with novel methods often being required to give an ecological assessment of these waterbodies. Chapter five gives more detail as to how these scoring metrics were constructed and how the samples were analysed.

Seed bank sampling

A sample of the seed bank was collected from each site using a coring device of 6 cm diameter and 60cm length. This was pushed into the soil at a random location on the plant and invertebrate transect route, to the maximum depth possible. The emergent zone was sampled for all sites, due to more efficient coring and a reduction in variability caused by including submerged soils (Yuan *et al*., 2007). The core was removed from the coring device and

wrapped in aluminium foil to exclude light. Any large pieces of surface vegetation were removed before wrapping. The cores were bagged and labelled on site, and stored in a refrigerator at approximately 2°C.

After approximately 2 months in storage, the cores were removed. The soil cores were washed through tiered sieves of mesh size 10 (1.68 mm) and 60 (0.251 mm) in accordance with studies of other wetland seed banks (Ter Heerdt *et al.*, 1996). The material on the lower tiered sieve was collected, and added to tubes containing approximately 10 g of sodium carbonate. Chemical floatation methods were used (Michigan State University, date unknown, Mesgaran *et al*., 2007), causing lighter organic components to float to the surface. These were skimmed from the surface of the solution by hand.

The collected component was placed into standard plastic Petri dishes and dried in an electric propagator at approximately 30 °C for 48 hours, with lids removed. Once fully dried, the contents were disturbed using a bent mounted needle, to remove any adherence to the petri dish. Air flow separation was then carried out by a gentle blowing and oscillating technique. This removed the light-weight components of the organic/seed mix, whilst retaining the seed components and any heavier organic components. The removed reside was checked routinely to ensure no seeds had escaped during air flow separation.

To enable accurate identification of the seed types and numbers, samples were hand sorted to divide the seeds into different categories. Identification of the seeds to species level was not possible due to a lack of suitable seed keys for riparian and wetland species. Seeds were therefore categorised using visible identifiable differences. This method has previously been used to reflect the diversity of different species seed types (Martin and Barkley, 1961). A spotting well was used to hold the separated seed types. A list of physical descriptions was developed during the process to aid with sorting. Timings for this stage of the process varied, with an average of 30minutes being required per sample. Identification was aided with use of a low power digital microscope attached to a monitor, as well as a hand lens. Once separated, seeds were stored in small plastic bags. These were labelled with site details, seed type from the key, seed numbers and any other observations, and retained for future use.

Identification by the sieving/flotation technique was deemed more applicable to this study than the seedling emergence technique (Roberts, 1981). The seedling emergence technique requires seed samples to be germinated under a standard set of conditions. Due to the variability in germination requirements for wetland species (Roberts, 1981), this method was

thought to be more likely to give false results through variability. High levels of variance have been illustrated in other studies using this method (Langdon *et al*., 2004; McFarland and Shafer, 2011). Pierce and Cowling (1991) compared both seed counting and germination methods, with little difference between the two.

Water Sampling

The water samples were collected in 150ml new plastic bottles that were triple washed with distilled water. They were further washed by triple washing with the sampling location water, before the main sample was collected. Approximately 100ml of water was collected. Samples were filtered after collection, and frozen for later analysis.

In field measurements of pH, conductivity and particulate matter was measured using a Griffin Model 50 pH Meter and a HM Digital Model Com-80 meter (for conductivity and particulate matter). Both meters were regularly recalibrated using known standards, in accordance with manufacturer's guidelines.

The laboratory analysis of the water included measures of nutrients and metals. A Burkard Series 2000 auto-analyser was used to measure total organic nitrogen, phosphates and ammonia. A Jenway PFP7 flame photometer was used to measure sodium and potassium. Chapter 7 gives a detailed account of the subsequent analysis of the water samples.

The following chapters examine the data that was collected in more detail, with a range of data manipulation and analyses. Chapter's four to seven address the four defined areas of plants, invertebrates, seed banks and water chemistry respectively in their own separate chapters, in an attempt to find whether an impact caused by the growth of *C. helmsii* could be found.

Chapter 4 – The Effects of *Crassula helmsii* on Macrophyte Assemblages

Introduction

The processes that underlie invasion impacts on plant communities are complex and often poorly understood (Emery and Gross, 2007; Gooden and French, 2015). Plant invasions can lead to a loss in native plant diversity (Leach, 1999; Fierke and Kauffmann, 2006; Michelan *et al.*, 2010; Andreu *et al.*, 2011). These losses could be caused by mechanisms such as direct competition (Gerber *et al.*, 2008), propagule pressure and vector delivery systems (Fierke and Kauffmann, 2006) and poor management decisions being taken (Burke and Grime, 1996; Kimball and Schiffman, 2003; Dostal *et al.*, 2013).

The idea of species loss due to invasion can however be challenged. Invasive species may not always be detrimental towards native species (Denoth and Myers, 2007). Poor experimental design may account for some of the examples of species loss by invasions (Wardle, 2001). Changes over time may also show very different results, with initial detrimental impacts changing after prolonged presence of an invasive (Dostal, 2013). In their study of invasive plant species, Bernard-Verdier and Hulme (2014) found that only 10% of the alien species that they studied caused statistically significant declines in species richness.

Species assemblage changes after invasion may also be scale-dependent. On a small scale data may indicate species loss, whilst at landscape level species losses may not be observable (Michelan *et al.*, 2010). Powell *et al.* (2013) showed how differences exist when examining invasive-mediated reductions in diversity on a smaller scale of less than 25 m^2. When this was compared to areas at landscape level, no evidence of a reduction in species diversity was found.

The effects of non-natives vary widely between different species. Hejda *et al.* (2009) found different invasive species had different effects upon native species compositions dependent on growth morphology and rooting systems. With the establishment of a non-native species, a positive feedback mechanism can occur that further facilitates changes to species composition (Green *et al.*, 2011), called invasion meltdown. Invasion meltdown is a process that can occur when an invasive species is found to facilitate the introduction and establishment of other invasive species (Grosholz and Tilman, 2005). Invasive species that have remained at low levels in a habitat may rapidly increase after an interaction with a new invasive species. This was

found to occur with the competitive release of the clam species *Gemma gemma* (Eastern Gem Clams), after the introduction of the non-native crab species *Carcinus maenas* (European Green Crab) in eastern U.S. coastal waters. Facilitation between non-native plants and animals may also occur in the form of vector transportation or pollination effects (Simberloff and Von Holle, 1999). Interactions that facilitate the establishment of non-natives between plants and birds, through food provision and subsequent seed dispersal have also been shown (Mandon-Dalger and Clergeau, 2004). Facilitation of establishment between invasive species has been shown not to always be true however, with studies available that illustrate facilitation between invasives often being over a short time period (Simberloff, 2006). The term 'invasion meltdown' is also criticised, and thought to be used by the media rather than scientific studies (Simberloff, 2006).

Species extinctions are often cited as a possible consequence of invasion, but little evidence exists to support this idea. In a comparison of IUCN Red List Species, only 6% were shown to be at possible risk from invasive species, whilst 33% were shown to be at risk due to habitat loss (Guevitch and Padilla, 2004). These two risks to species loss often occur in conjunction, which makes identifying the impact caused by invasive species difficult to evaluate.

It was traditionally thought that high species diversity makes a habitat more resilient to invasion (Elton, 1958). A study of *Ascidian spp*. (Sea Squirts) in the marine environment found a decrease in native species cover, followed by an increase in non-native species cover (Stachowicz, 2002). This has also been shown to occur in terrestrial systems, where invasive grass species have been shown to be limited by more diverse native macrophytes assemblage (Michelan, 2010).

The theory of invasion resistance has previously been challenged, with research showing that native species and invasive species can increase positively together (Stohlgren *et al.*, 1998). A comparison between island sites that supported three times the number of invasive species compared to mainland sites was carried out (Lonsdale, 1999). Island sites were found to have a comparable level of native diversity to the mainland (Lonsdale, 1999). Highly diverse systems have been shown to be stochastic, energetic systems with the likelihood of species rotations being high as one species is lost and another replaces it (May, 1973; Huston and DeAngelis, 1994; Piers and Johnson, 2004; Von Holle and Simberloff, 2005; Capers *et al.*, 2007). Species rotations may allow non-native species to enter a system, and so be positively correlated with greater diversity.

Inland, static waterbodies are an understudied habitat, with little regular data collection or monitoring (Williams *et al.*, 2003), with the majority of studies taking place on streams, rivers and lakes. Aquatic macrophyte diversity is generally lower in ponds and ditches than rivers and streams. Ponds and ditches can however still contain rarities that the rivers and streams do not (Williams *et al.*, 2003). Lentic systems are also an important factor in habitat wide diversity measures, acting as stepping stones between the larger catchments. Though these species pathways may initially seem beneficial to landscape scale diversity, increasing species movement of native macrophytes is also likely to encourage invasive dispersal. Macrophyte species richness has however been shown to correlate positively to the number of neighbouring waterbodies within a 500 m radius (Oertli *et al.*, 2002). Small, temporary ponds and ditches are capable of acting as biodiversity rich areas, capable of supporting species that are unable to thrive in the larger, permanent systems (Cereghino *et al.*, 2008). Temporary ponds were shown to make up 40% of lowland ponds within Britain in the Lowland Pond Survey (Nicolet *et al.*, 2004).On a scaled measure of the larger, more well studied systems, temporary ponds are often more diverse than their permanent comparatives (Cereghino *et al.*, 2008). It has also been shown that a collection of smaller ponds has a greater rarity value (more rarities present) than a similar combined sized single pond (Oertli *et al.*, 2002).

Aims

The aim of the macrophyte study was to investigate whether invasion by *C. helmsii* had a negative effect on macrophyte species richness on a range of sites in Kent, South East England.

Method

Locations supporting *C. helmsii* were compared with sites where it was absent (control sites). Further comparisons between habitat types were carried out, to ascertain whether *C. helmsii* had differing effects dependent on the habitat where it was present. Different waterbody types of ponds, lakes and ditches were analysed to determine whether *C. helmsii* had differing impacts dependent on the type of waterbody where it was present. Management was compared between actively managed sites, or those where it was either unmanaged or managed only through non-destructive methods of removal by hand. In a similar manner to

habitat and waterbody type, this was to provide a comparison between the possible effects of *C. helmsii* presence when combined with management as a secondary variable.

Plant species lists for each site visited were compiled in accordance with the methods stated in Chapter 3 – Survey Methodology. The results were scored using 5 different scoring systems for rarity. These were:-

- The Online Atlas of the British and Irish Flora (ATLAS – Biological Records Centre, 2013)
- The Botanical Society for the British Isles (BSBI, 2013)
- The Predictive System for Multimetrics (PSYM)
- The Atlas of Kent Flora (Philp, 1982)
- A New Atlas of Kent Flora (Philp, 2010)

The scoring system for each of these five systems provided a measure of rarity. The three national systems (ATLAS, BSBI and PSYM) measure rarity by the number of 10 km x 10 km hectads where the species is present. The county level scoring systems (Philp 1982; 2010) measure rarity in the same way, but by using 2 km x 2 km tetrads, due to the increased level of surveying detail.

The method of using rarity scores allowed for species compositions to be calculated. For example, *Urtica dioica* (Stinging nettle) is a common species, being present in a large number of hectads across the country and therefore returns a high hectad presence score. This inversely gives it a low rarity score. The opposite of this would be a species such as *Orchis purpurea* (Lady Orchid), which is an uncommon species not present in many locations and so would return a low hectad presence score. This therefore gives it a high rarity score. This was the same system used for the county level scoring metrics, but with tetrads replacing hectads as the measure of distribution. In this study, rarity is used as a representation of the data only, and should not be taken to provide a weighting towards desirability or conservation aim. The term 'conservation score' is often used interchangeably with that of rarity score to mean the same thing, but will be avoided throughout this thesis.

The PSYM method provides scores for just aquatic macrophytes, and so does not give a true representation of riparian drawdown margins that were included in the surveys and subsequent plant lists. These were included however, to ascertain whether they related to the other scoring systems that provided data for all plant species recorded.

The two Kent scoring systems by Philp (1982; 2010) are based on data derived just from Kent, and so used to give a county level score. These were included to give a more localised scoring system, and so give a more accurate representation than the national databases.

The national systems are based on hectad representation of presence of the plants, giving a score based on the total number of hectads. The ATLAS system gives scores independently for Great Britain and Ireland, and so scores just for Great Britain were selected. The BSBI system scores for both Great Britain and Ireland. The Kent based systems of Philp are constructed form tetrad level records. In each example, a higher number is representative of a more common, less 'rare' species.

Each plant species was scored in response to the metrics used, and then ranked dependent on the total number of hectads or tetrads from the full data set to scale the data. The ATLAS and BSBI scores were ranked from 16 (rarest) to 1 (most common), with a 250 hectad separation between each scoring integer and a maximum hectad available score of 4000. The PSYM method is scored by the procedure provided by the National Pond Survey methodology on a 1-32 doubling basis, with the higher number being representative of a rarer species.

Both Kent tetrad systems were ranked on a 1 to 21 scoring system, at 50 tetrad divisions between rank integers. This was based on a total tetrad score for the county of 1043. For this system, a score of 21 was representative of the rarest species, with a score of 1 being the most common.

Total rarity scores were calculated for each survey location. Average rarity scores were found based on the total score divided by total species numbers recorded. It was felt important to include these further measures, as it has been shown that total species richness on their own can lead to underestimation of alien plant impacts (Bernard-Verdier and Hulme, 2014). Each site therefore had three scores per scoring system, with a total number of 15 individual scores per survey location.

$$Average\ rarity\ score = \frac{Total\ rarity\ score}{Total\ species\ number}$$

Equation 1. Calculation of the average rarity score for each survey location, using the database rarity scores and measured species numbers from each location surveyed.

To ascertain whether *C. helmsii* was having an effect on native flora, sites were divided into invaded and clear (control) sites. Data analysis was by Mann Whitney analysis, with sample numbers of n=57 for *C. helmsii* sites, and n=21 for control sites.

Sites were divided into either 'Coastal', 'Lake' or 'Woodland' sites dependent upon the surrounding landscape type and proximity to neighbouring habitats. These were referred to as 'habitat comparisons'. Only average plant rarity scores were used for these comparisons, with total rarity scores and total species numbers excluded. These designations were compared to their partnering control sites by Mann Whitney analysis. This resulted in sample numbers of:-

- Coastal *C. helmsii* n=22
- Coastal control n = 5
- Lake *C. helmsii* n=15
- Lake control n=5
- Woodland *C. helmsii* n=11
- Woodland control n = 5

To analyse the difference between rarity scores for each waterbody type, into constituent locations of 'Ditches', 'Lakes', 'Ponds 1' and 'Ponds 2' (a sub-division of the pond data set was deemed necessary to equalise the sample numbers). This grouping of data gave numbers of

- Ditch n = 18
- Lake n = 6
- Pond 1 n = 13
- Pond 2 n = 20

Comparison was carried out by Kruskal Wallis analysis between the *C. helmsii* sites.

In order to represent the effects of management on *C. helmsii* sites, the rarity site scores were divided into actively managed sites, and unmanaged/target managed sites. Active management was categorised as evidence of recent spraying, excavation or shading control. Non- managed/target managed sites were categorised as no recent (within 5 years) management control, or control techniques that were specific to *C. helmsii* only. This was limited to physical removal by hand. This provided data sets of:-

- Active management n = 34
- Unmanaged/target managed n=23.

Comparison between the two datasets was by Mann Whitey analysis.

Plant diversity lists were used in the analysis of abiotic factors using MAVIS software. MAVIS allows for species lists to be used to generate a predicted score for light, wetness, pH and fertility dependent upon the species present. A further set of scores was therefore created and analysed in an attempt to ascertain whether *C. helmsii* had an effect upon plant diversity. The MAVIS system of analysis was used in the same manner as for the direct plant presence and scoring methods. The returned values were analysed in the same manner as the primary plant data, with 3 Mann Whitney tests and 1 Kruskal Wallis analysis per site.

Results

Waterbody Comparison

Comparison of water body type by Kruskal Wallis analysis found that all rarity scoring systems were significantly different when analysed - ATLAS (p=0.001), BSBI (p=0.001), PSYM (p=0.002), Kent 1982 (p=0.010), Kent 2010 (p=0.024). For each scoring system, ditch systems showed the highest average rarity score. Lake systems were either second or third depending on which scoring system is used. Pond system collection 1 is shown to have the lowest overall average rarity score when compared to the other groupings.

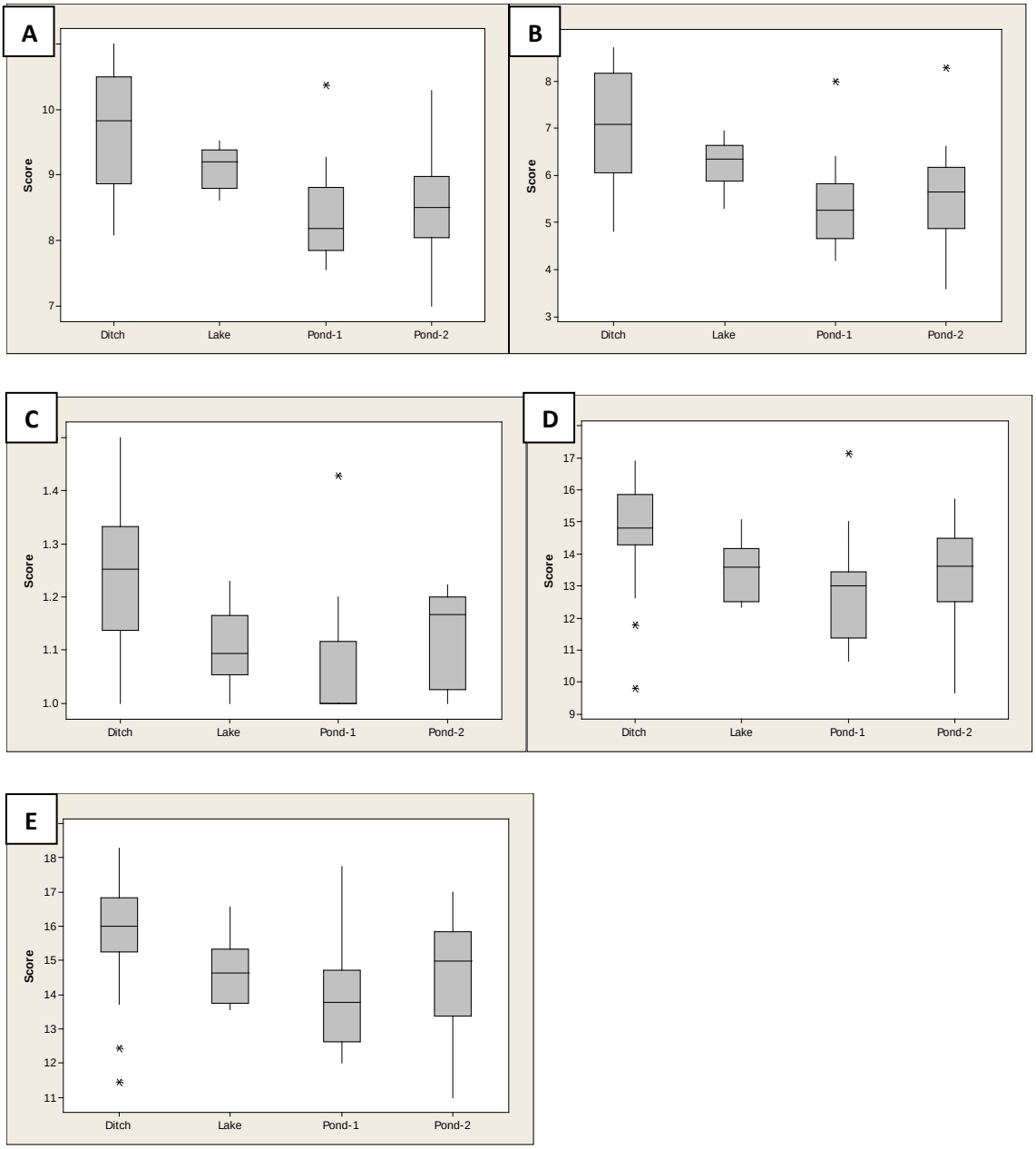

Figure 4.1. Boxplots of significant results from comparison between waterbody types of sites invaded by *C. helmsii*. A= ATLAS average rarity scores. B = BSBI average rarity scores. C = PSYM average rarity scores. D = Kent 1982 average rarity scores. E = Kent 2010 average rarity scores.

The Kruskal Wallis analysis of species number between the four separate water body types was found not to be statistically significant (p=0.2760).

Table 4.1. Probability values from data analysis of plant values, with significantly different results highlighted. The *C. helmsii* vs. control analysis compared invaded sites with control sites (Mann Whitney). The habitat analyses compared invaded and control sites, subdivided into the dominant landscape habitat type (Mann Whitney). The waterbody analysis included all of the study sites with no separation between invaded and control sites (Kruskal Wallis). The unmanaged vs. managed comparison compared management on *C. helmsii* invaded sites only (Mann Whitney).

	***C. helmsii* vs. Control**	**Habitat**			**Waterbody Type**	**Unmanaged vs. Managed**
		Coastal	**Lake**	**Wood**		
ATLAS Total Species Number	0.8565	0.0895	0.1007	0.9548	0.2760	0.4622
ATLAS Total Rarity Score	0.4537	0.0209	0.1901	0.8651	/	0.2513
ATLAS Average Rarity Score	0.0078	0.7788	0.2565	0.4278	0.0001	0.1431
BSBI Total Species Number	0.8432	0.0919	0.0809	0.9545	/	0.4622
BSBI Total Rarity Score	0.0932	0.0072	0.2938	0.8648	/	0.1875
BSBI Average Rarity Score	0.0126	0.6849	0.2947	0.7767	0.0010	0.2016
PSYM Total Species Number	0.3900	0.0443	0.1233	0.0619	/	0.0803
PSYM Total Rarity Score	0.3484	0.0351	0.0867	0.2725	/	0.1212
PSYM Average Rarity Score	0.4410	0.3467	0.8935	0.0605	0.0020	0.8309
1982 Total Species Number	0.8832	0.0899	0.1123	0.909	/	0.3605
1982Total Rarity Score	0.2111	0.0365	0.1903	0.0699	/	0.0938
1982 Average Rarity Score	0.0034	1.0000	0.2215	0.0065	0.0100	0.1618
2010 Total Species Number	0.8832	0.0899	0.1123	0.9090	/	0.3605
2010 Total Rarity Score	0.2070	0.0457	0.1904	0.0541	/	0.1165
2010 Average Rarity Score	0.0016	0.6619	0.1904	0.0092	0.0240	0.2318

Direct Comparison

There were significant differences between control and invaded sites for 4 of the 5 rarity scores -ATLAS (p = 0.0078), BSBI (p = 0.0126), Kent 1982 (p = 0.0034) and Kent 2010 (p = 0.0016) (Tab. 4.1). The box plots (Fig. 4.2) show that the higher rarity values are shown by the sites where *C. helmsii* is present.

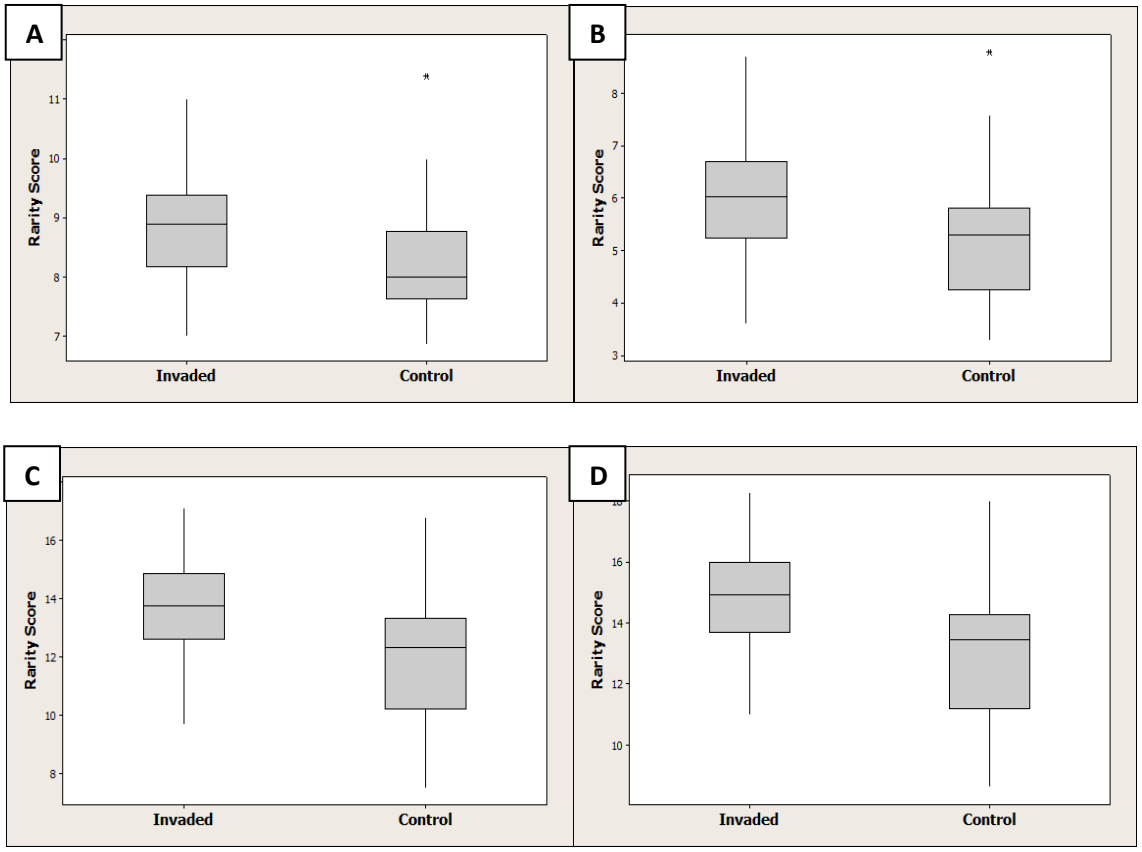

Figure 4.2. Boxplots of significant results of direct comparison between *C. helmsii* and control sites. The comparison shows average rarity scores of invaded and control sites. A = ATLAS average rarity scores. B = BSBI average rarity scores. C = Kent 1982 average rarity scores. D = Kent 2010 rarity scores.

Habitat Comparison

Analysis of the 3 habitat subdivisions of coastal, lake and woodland showed significant differences between invaded and control sites (Tab. 4.1). Coastal habitats comparisons returned significantly different results for total rarity scores for ATLAS (p = 0.0209), BSBI (p = 0.0072), Kent 1982 (p = 0.0365) and Kent 2010 (p = 0.0457) scoring systems, and total species number for PSYM (p = 0.0443). The box plots (Fig. 4.3) show that higher total rarity scores

were found on *C. helmsii* sites for the four scoring systems. The PSYM total species number was found to be significantly higher on the *C. helmsii* survey locations.

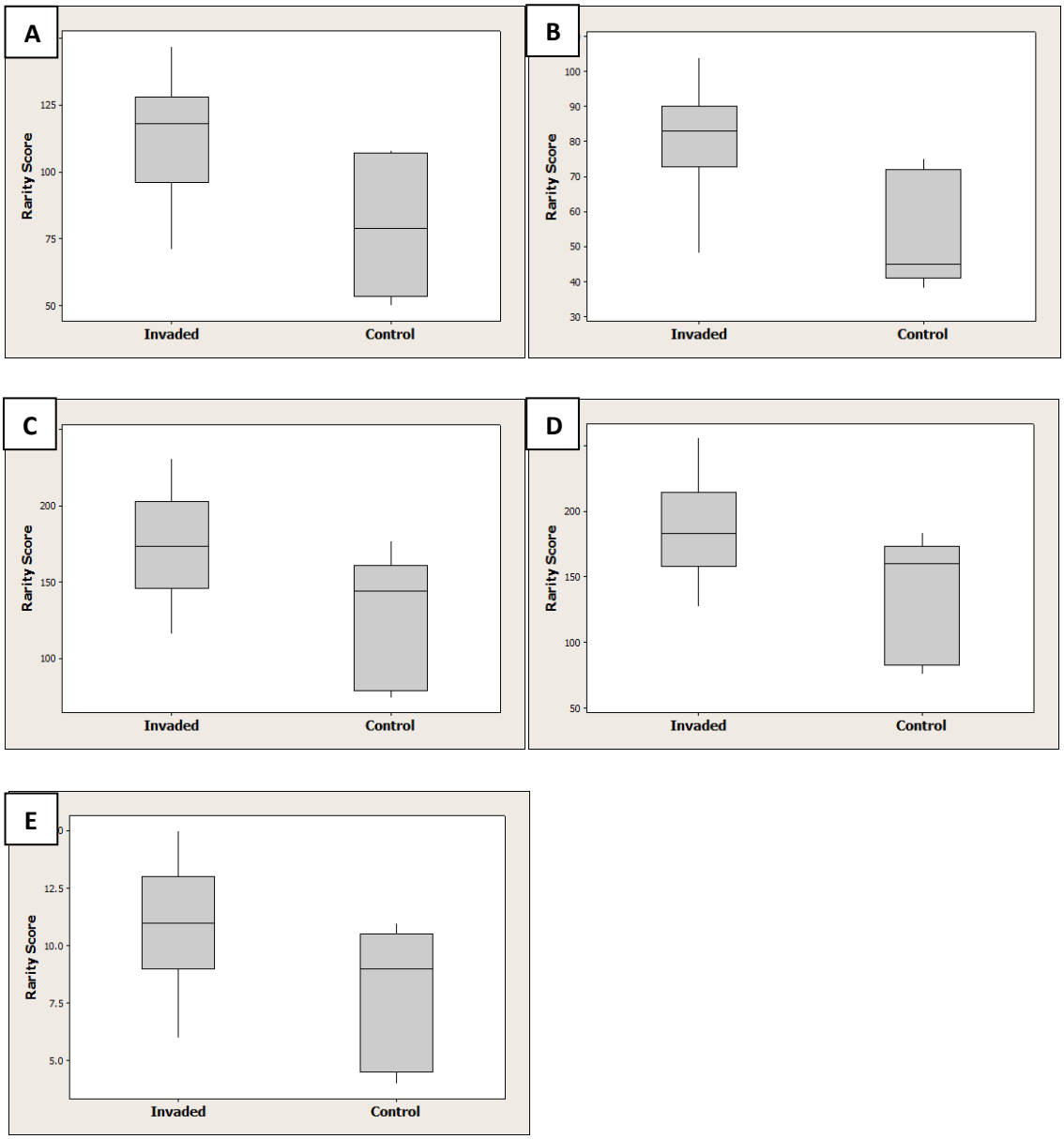

Figure 4.3. Boxplots of significant results from comparison between coastal habitat types. A = Coastal ATLAS total rarity scores. B = Coastal BSBI total rarity scores. C = Coastal Kent 1982 total rarity scores. D = Coastal Kent 2010 total rarity scores. E = Coastal PSYM total species number (diversity).

No significant results were returned for the lakeside comparison. For the woodland comparison, the Kent 1982 (p= 0.0065) and Kent 2010 (p=0.0092) average rarity scores were

found to be significantly different, with box plots (Fig. 4.4) showing that the higher scores were found on *C. helmsii* survey locations.

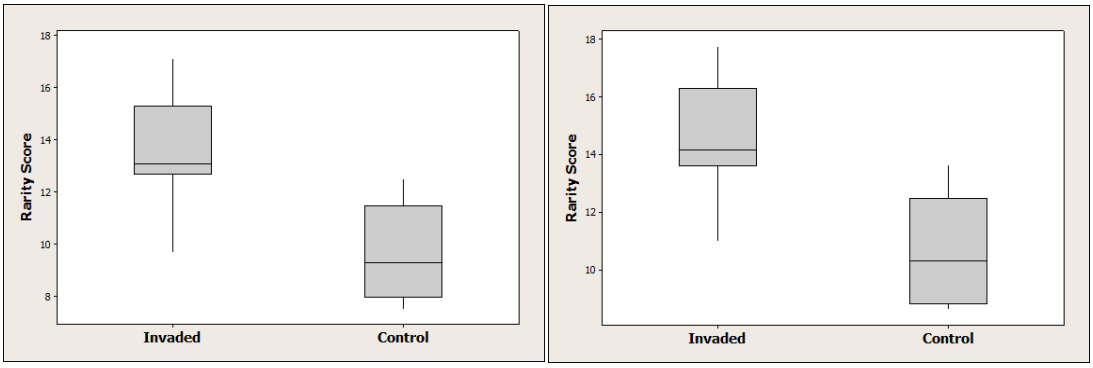

Figure 4.4. Boxplots of significant results from comparison between woodland habitat types. Left = Woodland Kent 1982 average rarity scores. Right = Woodland Kent 2010 average rarity scores.

Management Comparison

No significant difference in plant communities between managed and unmanaged/target managed sites was found.

MAVIS Analysis

Table 4.2. P-values of Mann Whitney data analysis, with source data derived from MAVIS using plant species lists.

	***C. helmsii* vs. Control**	**Habitat**			**Waterbody Type**	**Unmanaged vs. Target Managed**
		Coastal	**Lake**	**Woodland**		
Light	0.2118	0.5271	0.7913	0.8201	0.0140	0.6629
Wetness	0.7056	0.8380	0.2540	0.2552	0.0050	0.2098
pH	0.1676	0.9183	0.1115	0.6904	0.0580	0.6887
Fertility	0.9954	1.0000	0.0697	0.0674	0.0509	0.0352

The MAVIS analysis, which uses plant data to act as a predictor of four abiotic site factors, showed no significant results when compared directly between *C. helmsii* invaded and control sites, or for landscape habitat type. Statistically significant differences (Mann Whitney, p = 0.0140 and p=0.0050) were returned for light and wetness measures between waterbody type. Statistically significant results were found with the comparison of management and fertility (Mann Whitney, p=0.0352). Boxplot analysis showed that invaded sites that were managed had lower fertility levels than unmanaged sites.

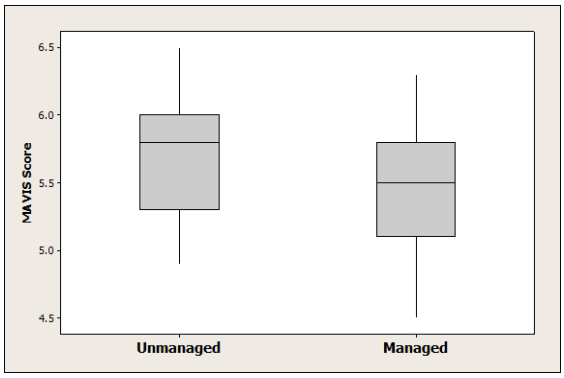

Figure 4.5. Boxplots of statistically significant results from comparison using MAVIS software analysis. MAVIS management comparison of fertility levels.

Discussion

Waterbody Comparison

The Kruskal Wallis analysis of the invaded habitats showed that significant differences existed between all waterbody types. This would appear to indicate that the creation of a monoculture after invasion by *C. helmsii* did not occur, and that the natural variation in species composition remained.

A study of waterbody types for macrophyte diversity found that natural variations do occur in species diversity, even when removing plant invasions as a variable (Williams *et al.*, 2003). In Williams' study, rivers (not included in the *C. helmsii* study due to its inability to grow in flowing water in natural systems) were the most diverse, with ditches being the least diverse but able to support rarities. In the *C. helmsii* study, ditch systems consistently scored the highest for rarity. Species numbers between sites were not found to differ significantly, and so this is only partially supported by the evidence. As rarity scores for sites have not been previously measured, it is difficult to judge whether invasion by *C. helmsii* has had an effect on

these systems, or whether it merely reflects the presence of greater numbers of rarer species in ditches. If it is considered with the comparison of invaded against control sites, it may be that it is showing evidence of invasion facilitating an increase in rarer species. Ditches, with naturally lower diversity, may be able to accommodate a greater number of species along with *C. helmsii*. This theory of increased exotics and increased natives co-occurring has been shown by previous studies (Stohlgren *et al.*, 1998; Smith *et al.*, 2006).

Ponds are known to be highly diverse systems, with a number of studies showing their significance (Linton and Goulder, 2000; Biggs *et al.*, 2005; Cereghino *et al.*, 2008). However, due to their ability to act as nutrient sinks for the wider landscape, they are often at risk to disturbance from resource fluctuations and sudden changes (Cereghino *et al.*, 2008). The *C. helmsii* study has shown that pond systems have consistently had the lowest rarity scores when compared to ditches and lakes. Previous research on ponds has shown them to be the most diverse of lentic systems (Williams *et al.*, 2003). This may indicate a larger impact on ponds than other lentic systems after invasion by *C. helmsii*, if the high diversity scores are assumed for this study. A possible explanation may be that high diversity in ponds did not prevent colonisation by *C. helmsii*, but was able to prevent colonisation by opportunistic native plants. This would be a modification of both the theory of invasion resistance not occurring (Stohlgren *et al.*, 1998), but with native species not benefitting from a change in the dominant flora (Von Holle and Simberloff, 2005; Capers *et al.*, 2007).

Direct Comparison

The results indicate that sites supporting *C. helmsii* have a significantly increased rarity score, and therefore 'rarer' species growing on them, in comparison to the *C. helmsii* absent control sites. The results of this study challenge the theory of species loss through invasion. Species loss was shown in a study of *Fallopia japonica* (Japanese knotweed), which was reported to cause significant decreases of native species richness after invasion (Greber *et al.*, 2008). Opposed to this, and supporting the *C. helmsii* data, is a study of riparian and upland habitats in the USA (Stohlgren *et al.*, 1998). This showed increases in exotic species and native species occurring simultaneously (Stohlgren *et al.*, 1998). Though the *C. helmsii* data does not show increases in species numbers, it does illustrate how species loss may not always follow invasion.

This study has shown that species numbers have not significantly decreased due to invasion by *C. helmsii*, but the average rarity score of the species present on invaded sites has increased. This would seem to indicate a change in species composition, towards rarer species on the invaded sites. Rodriguez (2006) suggests mechanisms as to how this may have occurred, of which habitat modification and competitive release may be applicable to *C. helmsii*. Habitat modification may be achieved by the addition of both structures for adherence of new species, or sheltered areas that allow for growth of macrophytes that may not have been present without *C. helmsii* biomass being present. This has been shown to occur for *Spartina alterniflora* (Smooth Cordgrass), which stabilises cobble beach habitats, thereby reducing disturbance and facilitating the growth of *Suaeda linearis* (Annual Seepweed) and *Salicornia europaea* (Common Glasswort) (Bruno and Kennedy, 2000). This may occur with *C. helmsii*, whereby sheltered areas create catchments for floating species such as the *Lemna* spp. (duckweeds), and *Hydrocharis morsus-ranae* (Frogbit), which may otherwise have been dislodged due to wind disturbance. *Lemna minor*, *Lemna trisulca* and *H.morsus-ranae* were recorded at some survey locations in this study, and so may explain the possible increases in rarity scores with *C. helmsii* present.

Competitive release of rarer species due to the reduction of a dominant native species may have occurred (Rodriguez, 2006), which would also facilitate the growth of *C. helmsii* (Emery and Gross, 2007). The ability for non-native species to alter species compositions in favour of rarer species and thereby create more diverse habitats has been exploited in ecological restoration (D'Antonia and Meyerson, 2002, Zarnetske et al., 2013). This release from competition by dominant native plant growth may be due to trophic interactions (Wonham *et al*., 2005). A study of riparian macrophytes found that natives and non-natives were able to exploit nitrogen deposits on an equal basis (Bradford et al., 2007), and not competitively exclude each other.

The response by non-natives to environmental changes has been shown to vary between different species, with some being a passenger to change rather than the genesis of change itself (Didham *et al*., 2005, MacDougall and Turkington, 2005, Bernard-Verdier and Hulme, 2014). These environmental stresses have been shown to have varying effects on both natives and non-natives (Turkington and Bradfield, 2006), and are dependent on the species and habitats being studied (Woitke *et al*., 2002, Didham *et al*., 2007). Whether an environmental stress has occurred as a precursor to loss of native dominance or whether invasion by *C. helmsii* was responsible for the decline in dominance is not clear from this study. MacDougall

and Turkington (2005) suggest an appropriate method of testing this 'passenger' theory, with the removal of the invasive resulting in the increase in diversity of other, novel native species. This is likely to be a difficult procedure to replicate for *C. helmsii*, due to the difficulty in removing the species (Dawson and Warman, 1987), but may help to provide evidence for the reason why it has colonised successfully.

This interaction between non-native species and rarer species has been shown to have a mutualistic response in other studies (Harris *et al*., 2004, Denoth and Myers, 2007). Invasive species may not be detrimental to rarer native species. *Lythrum salicaria* (Purple loosestrife), invasive in Canada, was shown not to reduce the growth of *Sidalcea hendersonii* (Henderson's mallow) over a 20 year study period (Denoth and Myers, 2007). *Ulex europeaus* (Gorse), a common and native species in the UK but an invasive species in New Zealand, has been shown to promote the growth of some groups of species in New Zealand. This resulted in increased species richness when compared to uninvaded control survey sites (Harris *et al.*, 2004).

C. helmsii was found not to lower native species numbers in this study. Though the ATLAS, BSBI and PSYM scoring systems are national, the Kent Atlas systems are county based, and so cannot be translated outside of the county to different sites. The effects on other survey locations outside of Kent may differ, as distribution records show *C. helmsii* to be more strongly associated with the south east of England (BSBI Maps, 2015). Invasives have different effects across different countries. A study of *Impatiens glandulifera* (Himalayan Balsam) in the Czech Republic showed that it had little effect upon native community characteristics and species composition , but in the UK *I.glandulifera* has been shown to have detrimental effects towards native species composition (Hulme and Bremner, 2005). The invasive species *Heracleum mantegazzium* (Giant Hogweed) caused an impact to native plant species, on the same survey sites where *I.*glandulifera was shown not to have an impact towards natives (Pysek and Pysek, 1995; Hejda and Pysek, 2006). These differences were thought to relate to the morphology of the plant species, and the ability to compete for light more successfully (Hejda *et al.*, 2009).

Habitat Comparison

The habitat comparison of invaded and control sites showed significantly different results for total rarity scores on coastal habitats for ATLAS, BSBI, Kent 1982 and Kent 2010 scoring systems. The PSYM scoring system showed a significant result for species number on coastal

sites. Each of these results showed significantly higher levels on *C. helmsii* invaded sites compared to the controls.

The PSYM methodology did not show any significant difference between invaded and control sites in the direct comparison. This may be due to only aquatic and not riparian species being included in the PSYM scoring system. As *C. helmsii* is able to grow across a range of habitat morphologies, not including the full range of riparian species may not provide an accurate representation of the in-field situation. The PSYM score of increased species number on coastal *C. helmsii* sites in the habitat comparison may be due to the limitations of the scoring system which does not include terrestrial species. The majority of coastal sites surveyed were ditch systems with a diverse terrestrial species composition upon the bankside habitat (Oare, Rye Street, Dungeness and Rye Harbour were all predominantly ditches). The significantly different total rarity scores for the other 4 scoring systems may be due to species numbers being lower on invaded coastal sites compared to coastal control sites.

Any changes to species composition would therefore have had an amplified effect on total rarity scores on invaded sites compared with control sites. The average diversity scores were not found to be significantly different between invaded and control coastal sites. It may be therefore that these results are a reflection of low initial diversity, which was susceptible to statistical change due to *C. helmsii* being included in the analysis. It may also be due to the ability for *C. helmsii* to alter the chemical component of the water bodies after invasion. It is known to have the ability to accumulate heavy metals (Küpper *et al.*, 2009). If this accumulation extends to other components of saline water, it may allow plant species to grow here that would not have been able to pre-invasion. Further investigations of a larger range of metals and nutrients would be required. The modification of the studied waterbodies' chemistry is commented on further in Chapter 7.

Woodland habitat results indicated an increase in average rarity scores for the county level scoring systems. As this is limited to just Kent, and was not found for the other scoring systems, it may be a regional effect, and so would require further studies outside of the county to investigate regional differences. The reduction in light levels at the woodland sites may have limited growth of *C. helmsii* due to a limitation of photosynthetic activity. Though it is able to grow under low light levels (Hussner, 2009), its ability to use the CAM system of photosynthesis is better utilised under high light levels (Newman and Raven, 1995; Klavsen and Maberly, 2010). The fact that control ponds had a lower average rarity score may be descriptive of a dominance of native flora preventing invasion and subsequent opportunistic

native species, which would lead to increases in the average rarity score of the site. This is different to previous studies of *C. helmsii,* where species losses were thought to occur (Leach, 1999). A wider ranging study found no loss in macrophyte species numbers (Langdon, 2004), but gave no description of the macrophyte composition of the sites being studied.

Management Comparison

This study of *C. helmsii* has found no link between loss of plant species and management practices. This would initially seem encouraging, but the long term effects of untargeted control methods where all species in a target area are removed are unknown. This may have implications with a reduction of native plant species due to adverse impacts towards the seed banks. This will be explored in more detail in a Chapter six.

The method of control is a critical decision when managing impact towards native plant species. The invasive grass *Microstegium vimineum* (Japanese Stiltgrass) was found not to have any measurable effect on native flora species richness, with the main reason for species loss being due to poor management (Brewer, 2010). Native species were found to be adversely affected by artificial clipping (used to mimic grazing), whilst invasive alien species showed no detrimental growth effects, on a Californian grassland (Kimball and Schiffman, 2003). When this was repeated for European grass species (which have evolved under higher levels of grazing pressure) different effects were found, with natives not being affected in the same manner. It is therefore important that management decisions be taken under a theory based and preferably trialled system. The difficulty with this approach is that no universal method is likely to establish, due to the complexity that different invasives show on different habitat (Pysek and Pysek, 1995; Hejda and Pysek, 2006).

MAVIS Analysis

The MAVIS analysis showed differences between waterbody types, which are consistent with previous results between waterbody comparisons using the plant based scoring measures. The variability between the score of wetness (a measure of length of submergence and/or soil saturation) and light levels are likely to reflect the difference between waterbody types, and likely to represent normal variation between waterbodies. The difference in fertility between management types may expose a detrimental impact of management. Topsoil removal is often

used in non-target control methods, which would include removal of nutrient. This may have resulted in macrophyte assemblages being present that represent lower nutrient presence.

Further factors could be considered when examining the data and statistical output. The idea of habitat scale of the investigation may be important. Experiments have shown that small scale changes are not always represented on a landscape wide basis. A study of three invasive plants; *Dianella ensifolia* (Cerulean Flax Lily), *Lonicera maackii* (Amur Honeysuckle) and *Morella faya* (Fire Tree) were all shown to cause local decreases in macrophyte diversity (Powell *et al.,* 2013). When examined on a landscape scale and compared to control sites, no significant difference in species loss between invaded and controls could be found.

The length of time that an invasive species is present on a site is also likely to be an important factor. It has been shown that the effect of an invasive macrophyte species decreases over time. Dostal *et al.* (2013) showed that the effects of *Heracleum mantegazzianum* (Giant Hogweed) decreased between a 48 year separation in sampling time. A decrease in impact by invasives over time was also shown in a study of *Phalaris arundinacea, Rubus armeniacus and Hedera helix* (Reed Canary Grass, Himalayan Blackberry and English Ivy) (Fierke and Kauffman, 2006). Morphological and physiological changes of native species may account for this decrease in the effect of invasive species, but require a prolonged selective pressure of invasion to facilitate change (Strayer *et al.*, 2006). The time separation of the Kent scoring systems is only 28 years, and so may not show this change. It may be that the scoring system method will indicate how habitats change due to invasion over time. This will require new updated scoring systems in subsequent decades to be developed.

The discrepancy between the scoring systems illustrates a flaw in using scoring metrics that are not continually updated. The PSYM method and its scoring metrics are, at the time of writing, more than 13 years old (Howard, 2002). The BSBI and Kent 2010 scoring metrics were more recently constructed, with BSBI scores renewed every 2 years (BSBI, 2014). Whilst the Kent 1982 and Kent 2010 have returned similar results throughout, the national scoring metrics would likely experience far greater changes to in-field situations. The creation of a digital scoring system that utilised up-to-date scores, as opposed to paper based methods such as PSYM, would likely encompass far greater levels of accuracy when looking at national trends of distribution. Data integrity is important for decision making in conservation and for making

management decisions, with the accurate representation of species distributions shown to be a key factor (Grand *et al*., 2007).

Conclusion

Though species numbers do not change significantly when comparing invaded and uninvaded sites, species composition does. Average species rarity scores of invaded sites have been shown to increase when compared to control sites. The mechanism for this has been suggested as a reduction in competition from the dominant native species, which not only facilitates invasion by *C. helmsii*, but also promotes other native species to occupy the habitat alongside it. This results in an altered composition of plants, but not a reduction in numbers. The idea of 'rarer' species being present is not necessarily a good thing. If a habitat is being managed to retain a particular composition that is desired, the change towards rarer species may be a negative factor of *C. helmsii* presence. There may also be benefits of having rarer species. They may be able to support a wider range of species through the provision of food and shelter that would otherwise have been lacking.

It has been demonstrated that *C. helmsii* has had different impacts on habitat and waterbody types. The coastal system showed a significant change in composition. This may be a reflection of initial low diversity making the scoring systems more susceptible to change. Woodland habitats which provide tree cover and shading may show the effect of low light levels reducing growth due to the inability of CAM photosynthesis to be fully utilised by *C. helmsii*. Ditches have been shown to have high rarity scores compared to previously published values, which may suggest the establishment of rarer species within the plant assemblages of a habitat after invasion. Though management practices would appear to show no effect towards macrophyte assemblages, this is a factor that should be monitored over time, as future results from field studies may reflect a different response to the comparison. The MAVIS analysis has indicated a possible impact of nutrient removal by untargeted management, which is reflected in the species assemblages.

Chapter 5 – The Effects of *Crassula helmsii* on Freshwater Macroinvertebrates

Introduction

The growth of non-native aquatic plants is likely to have effects on the composition of other species groups beyond that of macrophytes. Freshwater ecosystems consist of many species groups, with interactions occurring between different biotic and abiotic systems (Kneitel and Leissin, 2010; Ormerod *et al.*, 2010). Freshwater invertebrate are one of these groups, with all or part of their life cycle stages often occupying an aquatic stage (Quigley, 1977). Phytophagous invertebrates may account for a considerable percentage of the energy flow through the secondary producer trophic level (Gerber *et al.*, 2008). Species invasion which result in changes to these species may therefore cause changes to the energy flow through the habitat. Changes to invertebrate diversity can be caused by alterations to macrophyte species (Valinoti *et al*., 2011). This has been shown to have effects upon other species within the aquatic system, such as fish (Villamagna and Murphy, 2010),

A comparative study of rivers, streams, ditches and ponds was conducted to ascertain the importance of these habitats (Williams *et al.*, 2003). It was found that pond systems had greater macro invertebrate assemblages, and supported more species than both rivers and streams. Ditches were found to be relatively species poor in comparison, but supported rarities not found in any of the other four freshwater classifications (Williams *et al.*, 2003). Similar results were found for temporary ponds. Temporary ponds receive little statutory protection, and are vulnerable to change by pollution and degradation (Nicolet *et al*., 2004). They are common across Britain, with approximately 40% of ponds studied in the Lowland Pond Survey of 1996 categorised as temporary (Nicolet *et al.*, 2004). Though less species rich than permanent ponds, temporary ponds are capable of supporting rarities not found in other waterbodies (Oertli *et al.*, 2002, Cereghino *et al.*, 2008). The size of a pond may not be an important factor for diversity. No relationship was found between size and macroinvertebrate diversity for *Coleopteran* and *Sphaeriidae*, as well as for *Amphibia* (Oertli *et al.*, 2002). This shows the importance of these static waterbodies as a component to ecosystem diversity. The inclusion of a range of waterbodies of varying sizes during surveying is therefore an important component of a surveying strategy.

The assumption that high macrophyte diversity is consistent with a high macroinvertebrate assemblage has been shown to not always be true. A comparison of Coleopteran (Water Beetle) assemblages and macrophyte assemblages was carried out to ascertain their effectiveness as a diversity indicator (Gioria *et al.*, 2010. The results provided evidence that a direct link between macrophytes and macroinvertebrates was not present (Gioria *et al.*, 2010). Ponds dominated by a single species, such as *Typha latifolia* (Greater Reed Mace), were still capable of supporting a diverse range of Coleopteran species.

Effects of invasion by macrophytes species on macroinvertebrates are present in the literature. Studies have shown that invasions may lead to increases in macroinvertebrates (Harris, 2004; Siersma *et al.*, 2007; Lecerf *et al.*, 2007; Tanner *et al.*, 2013). Examples also exist to show the opposite, where macrophytes invasions have caused decreases in diversity (Lecerf *et al.*, 2007; Gerber *et al.*, 2008; Hanula and Horn, 2011; Hladyz *et al.*, 2011; Tanner *et al.*, 2013). There are also examples where studies have shown no measurable effects on macroinvertebrates after macrophyte invasion (Braatne *et al.*, 2007; Siersma *et al.*, 2007; Stiers *et al.*, 2009; Bottolier-curtet *et al.*, 2011,).

These studies show that it is not clear whether plant invasions may be detrimental to macroinvetebrate diversity, with studies often showing two different results depending on the species studied or the macroinvertebrate sampled. It is unlikely therefore that a single rule on species loss exists that governs the link between invasions and species assemblages, with each case requiring specific study and consideration.

Previous studies on the effect of *C. helmsii* are sparse, with examples being either anecdotal from land managers or conservation bodies, or unpublished reports generated by keen amateurs. For example, Langdon *et al.* (2004) found that development times for *Lissotriton vulgaris (*Smooth Newt) were significantly later in invaded trials compared to controls. The overall impact on egg laying and neonate survival was, however, not significantly affected by *C. helmsii* invasion. No work was carried out on macroinvertebrates by Langdon *et al*., (2004), and so an examination of these effects was considered necessary.

Aims

The aim of the study was to examine whether any measurable effects of invasion by *C. helmsii* on freshwater macroinvertebrates existed. A comparison between macrophyte and macroinvertebrate measures on invaded and control study areas was also investigated. This

comparison was carried out to ascertain whether any interactions could be found these two groups, which may make estimating species diversity of macroinvertebrates more efficient.

Method

Freshwater macroinvertebrate samples were collected from waterbodies between May to August 2013. Sampling was carried out in accordance to the procedures as stated in Chapter 3 – Survey Methodology.

Table 5.1. Macroinvertebrate sample numbers from direct comparison between *C. helmsii* invaded sites and control sites.

Site Name	Samples from *C. helmsii* Sites	Samples from Control Sites
Oare	5	2
Gunpowder Works	0	3
Orlestone	0	2
Hothfield	1	4
Shorne Woods	4	0
Bough Beech	5	1
Sevenoaks	4	1
Perry Woods	2	0
Rye Street	5	2
Rye Harbour	5	0
Stodmarsh	5	2
Beacon Woods	2	0
Blean Woods	2	0
Eastry	2	0
Fowlmead CP	0	3
Total	**42**	**20**

Samples were fixed in formalin and transferred to IMS for long term storage. Identification of the specimens was carried out to family level. This was due to the requirement of this level of accuracy as prescribed by the scoring systems of PSYM and BMWP.

Three measures were used to measure impact. These were values for the number of different family groups present, total site score and an average site score (derived by dividing the two former measures). Analysis of the resulting groups was carried out by Mann Whitney analysis.

Relationships between habitat type and management activity were also considered. This was achieved by subdividing the data sets dependant on which of these groupings the sample was sourced from. Statistical analysis of the data was by Mann Whitney U analysis.

Table 5.2. Macroinvertebrate sample numbers from secondary comparisons.

	Site Designation	**Samples from *C. helmsii* Sites**	**Samples from Control Sites**
Habitat	**Coastal**	15	4
	Lake	9	5
	Woodland	10	5
Management Type	**Unmanaged/Target Managed**	18	N/A
	Active Management	24	N/A

Revised Scoring System

Most scoring systems for invertebrates are based upon the presence of certain family groups or species in polluted waters (Williams and Dussart, 1976). Scores are allocated at a family, group or species level accordingly, with less polluted water generally being the desired aim for waterbody conservation. Using these scores to illustrate invasion effects may be incorrect. Their use for small and often temporary lentic systems may not be applicable, with no accurate method existing for these waterbodies (Van den Broeck *et al*., 2015). In a review of 75 studies that used waterbody scoring metrics, Van den Broeck *et al*. (2015) found no evidence for a scoring system derived for the impact of non-native species on native species assemblages. Therefore a scoring system was devised that was more relevant to the particular impact of macrophyte invasion on invertebrate presence.

Data from the uninvaded control sites was segregated from the invaded site data. Macroinvertebrate family groups were given a score based upon the total number of sites that they were found to be present on, to give a measure of rarity. A ranking system was then applied to find the most common family groups. These were scored accordingly:-

- A rank of 1-5 = 0
- A rank of 6-10 = 1
- A rank of 11-15 = 2
- A rank of 16-20 = 3.

Total scores and average scores for each site were calculated.

$$ASSc = \frac{\Sigma \mathrm{FS}}{n}$$

Equation. 1 Calculation of average site scores using the modified scoring system. ASSc = Average Site Score, FS = Family Score, n=Number of family groups present.

This is similar to the use of Community Conservation Indices developed to show community diversity and not pollution effects (Chadd and Extence, 2004). Within the community conservation index a further multiplier, dependent on a rarity scoring system for the scarcest species present, was also used, but requires identification to species level.

$$CCI = \frac{\Sigma \mathrm{CS}}{n} \times CoS$$

Equation 2. The scoring of macroinvertebrates by community (Chadd and Extence, 2004). CCI = Community Conservation Index, CS = Conservation Score (Taken from Red Data Book Information), n = Number of different species present, CoS = Community Score (a single number representing the rarest taxon present, taken from Red Data Book or BMWP derived data).

The scores generated from the uninvaded control sites were used to score the invertebrate family groups on invaded sites. Any family group not present on control sites but recorded on invaded sites received a -1 score. A rating of invasion impact could therefore be created using the difference from the family groups present on the control sites. This gave a scale of 'naturalness', using the uninvaded sites as the method of generating reference scores.

The scoring system allowed for total scores, species number and average site scores to be created for both invaded and control sites. The number of sites analysed in this way was the same as for the revised BMWP method. These were compared using Mann Whitney analysis.

Macrophyte and Macroinvertebrate Correlation

A further investigation was compiled to find whether measures of macrophyte diversity could be used as an accurate predictor of macroinvertebrate diversity, both site diversity and species number scores were correlated. Linear correlation functions were used to determine whether any significant relationships existed between the two data sets.

Results

Comparison between *C. helmsii* invaded sites and control sites

The Mann-Whitney analysis of *C. helmsii* sites against control sites found no significant difference for species number, totalled site rarity or average site rarity.

Comparison between *C. helmsii* invaded sites and control sites, subdivided into habitat types

Habitat comparisons showed one significant difference between invaded and control sites. This was for revised BMWP on lake habitats (Mann Whitney analysis, p=0.0455). Boxplot analysis (Fig. 5.1) showed that the control sites scored significantly higher.

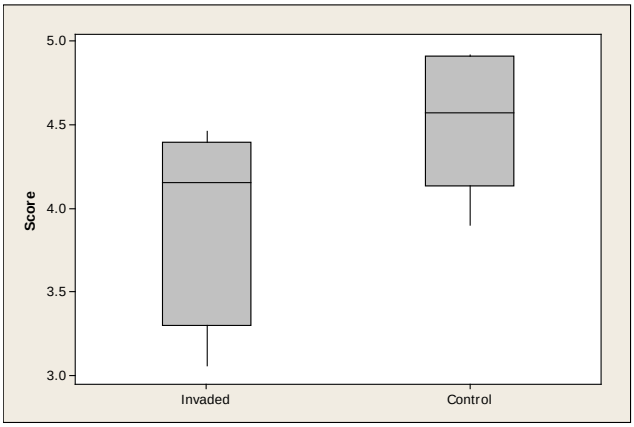

Figure 5.1. Boxplot analysis of comparison between macroinvertebrate (rBMWP) data from lake habitats. In this boxplot of comparison between invaded and control lake habitat sites, revised BMWP scores for average species rarity were higher on control sites.

Comparison between *C. helmsii* invaded sites and control sites, subdivided into management activity

The Mann Whitney comparison of data sub-divided into management activity of either active management or unmanaged/target managed found no statistically significant (p<0.05) results. This was the result for the individual measures of site species number, site total rarity score and site average score, as well as the summed data of macrophytes and macroinvertebrate comparison.

Revised Scoring System Results

The Mann Whitney analysis using the revised scoring system found no significant differences for total species number, total score or average site scores between invaded and control sites.

Correlation between macrophytes and macroinvertebrates on *C. helmsii* invaded sites

Linear correlation found no significant relationships between macrophyte and macroinvertebrate indices. Table 5.3 shows the resultant statistical output, with comparisons between species numbers and site average rarity scores.

Table 5.3. Results of linear correlation between macrophytes and macroinvertebrate comparisons of invaded sites. The correlation values and probability values (0.05) show no statistically significant results.

Macrophyte Scoring System	Macroinvertebrate Scoring System	Pearsons Correlation Value	Probability Value (P)
ATLAS Species No.	BMWP Species No.	0.040	0.803
ATLAS Average Score	BMWP Average Score	0.101	0.524
BSBI Average Score	BMWP Average Score	0.065	0.684
PSYM Average Score	BMWP Average Score	0.171	0.279
Kent 1982 Average Score	BMWP Average Score	0.168	0.286
Kent 2010 Average Score	BMWP Average Score	0.146	0.357
ATLAS Average Score	rBMWP Average Score	0.092	0.563
BSBI Average Score	rBMWP Average Score	0.058	0.714
PSYM Average Score	rBMWP Average Score	0.189	0.232
Kent 1982 Average Score	rBMWP Average Score	0.153	0.334
Kent 2010 Average Score	rBMWP Average Score	0.133	0.403

No significant correlation relationships were found between any of the data comparisons.

Discussion

Comparison between *C. helmsii* invaded sites and control sites

The direct comparison between *C. helmsii* invaded sites and control sites showed no statistically significant difference between the two. Therefore, from this data set it can be seen that invasion by *C. helmsii* has had no measurable effect on macroinvertebrates numbers or diversity. This is supported by studies of other macrophyte invasives, where no changes were observed post invasion. In a study of the effects on macroinvertebrate diversity after invasions by *Phragmites australis* (Common Reed) in the Great Lakes, it was shown that no significant

difference existed between invaded and uninvaded sites (Siersma *et al.*, 2007). No significant difference was shown for both macroinvertebrate richness and species composition. A study of the invasion of riparian margins by *Fallopia japonica* (Japanese Knotweed) found no significant difference between native litter and *F. japonica* litter when examined for macroinvertebrate colonisation (Bottolier-Curtet *et al.*, 2007). A UK study of the riparian invasive *Impatiens glandulifera* (Himalayan Balsam) showed no significant difference between invaded and control plots when compared for below ground dwelling macroinvertebrates (Tanner *et al.*, 2013). Three non-native species that can occupy the same niche as *C. helmsii*, and so can be seen as presenting similar effects towards native species are that of *Myriophyllum aquaticum* (Parrot's Feather), *Hydrocotyle ranuncluloides* (Floating Pennywort) and *Ludwigia grandiflora* (Water Primrose) (Stiers *et al.*, 2009). In a study of their effects towards macroinvertebrates in Belgian ponds, it was shown that though they did cause decreases in abundances of macroinvertebrates although no significant changes in diversity were found (Stiers *et al.*, 2009).

It has been shown that newts are able to utilise *C. helmsii* as an egg laying substrate (Langdon, 2004). Therefore, it would seem possible that macroinvertebrates may also be able to utilise the plant material. It has been shown that invasive species that provide additional litter and moisture at the ground layer have an additive effect towards macroinvertebrate diversity (Siersma, 2007). There may also be subsequent changes to detritivore species composition, which favour the littler layer (Dangles *et al.*, 2009). As *C. helmsii* can create a dense understory, it is likely that this could act as a microhabitat for macroinvertebrates, and provide both shelter and moisture. Whether macroinvertebrates can utilise *C. helmsii* as a food source is unknown. In its native habitat, it is known to eaten by invertebrate species, and is the source of locating a possible biological control agent (Varia, 2013). Whether any native species could utilise it is unknown. Preliminary work on *C. helmsii* using tank trials that offered *C. helmsii* to *Helix aspersa* (Garden Snails) has been attempted, but showed limited evidence for any grazing effects (Smith, unpublished work).

Comparison between *C. helmsii* invaded sites and control sites, subdivided into habitat and management

One lake habitat was found to have a significant difference (p=0.0455). This result showed that the control sites had significantly greater site rarity scores. This requires further investigation,

as why lake habitats may be more susceptible to change when compared to coastal and woodland habitats is unknown. Further studies of lakes that have been colonised by *C. helmsii* should be sourced and surveyed. As lakes are the largest waterbody type compared to the others surveyed, impacts may have come from many different inputs beyond just that of *C. helmsii* presence. It has been shown that boat traffic between lakes can cause non-native species to be distributed (Muirhead and Macisaac, 2005). Herbet *et al*. (1989) showed how lakes can act as indicators of long distance dispersal mechanisms for non-native species. This predisposition of lakes habitats to invasion and their mixed use for recreation and as a sink for dispersal may therefore have wider ranging effects on macroinvertebrates than smaller waterbodies, which are less likely to receive propagules.

The comparison between managed and unmanaged sites showed no significant differences in macroinvertebrate results. This would suggest that active management and removal of plant material is not impacting on macroinvertebrates species distributions. This may be due to other habitats being available that macroinvertebrates can disperse to, and so remain present at the surveying locations. This would lead to rapid recolonisation of both the waterbodies and riparian habitats after management had been conducted. It may also be due to management having no direct effects on macroinvertebrates, with sprays being targeted with little accumulative side effects, such is the case with glyphosate sprays (EPA, date unknown). Physical removal of plant material may not have large numbers of passenger species, and so losses in this manner may not be significant. It could also be due to the creation of more bare ground after management work, which is favoured by some invertebrate species (Croft, 1986, Atkinson *et al*., 2004) and so any losses would be counteracted by the appearance of new species. A previous study on the effects of management of *C. helmsii* on invertebrate diversity found no effects from management (Gardiner and Charlton, 2012). A system of continued monitoring post management would be required to better understand whether management remained targeted towards *C. helmsii*, or if the effects changed with successive management attempts.

Revised Scoring System

The creation of a scoring system that did not use pollution levels as a method of determining presence or absence on macroinvertebrates was deemed necessary, due to the likely difference between response to pollution and response to invasion. No significant difference

was found when comparing invaded and uninvaded sites. This indicates that *C. helmsii* had no measurable effect on macroinvertebrate assemblages when using this method of scoring index. This could be further explored, possibly using a continuation of Chadd and Extences' (2004) method of including a rarity multiplier within the equation, using the rarest species recorded. This would require identification to species level to be effective and further investigation of local databases for macroinvertebrate distributions. These would ideally not be linked in any way to pollution measures, to retain the desired distance from pollution based measures. As the results were the same as for the BMWP results, the creation of a scoring index to reflect the impact of *C. helmsii* and not one based upon water pollution are difficult to distinguish. A further exploration of other non-natives and their impact, and comparison of these results with more widely used metrics such as BMWP scores would be beneficial. Using a novel approach to scoring macroinvertebrates when measuring invasive species impact was not found to differ in this study, but this may not be the same result for other species.

Correlation between macrophytes and macroinvertebrates on *C. helmsii* invaded sites

The results show that a poor correlation existed between macrophytes and macroinvertebrates on *C. helmsii* invaded sites. Measuring site macrophyte characteristics does not act as a predictor of macroinvertebrate status. This is the same result for both species numbers and average site rarity scores. Macrophyte scores cannot therefore be used as a legitimate way of estimating the ecological diversity of a wetland, as has been proposed in previous studies (Awal and Svoziul, 2010; Gioria, 2010). Future studies of *C. helmsii* invaded sites, as well as impact studies of other non-native species, should therefore look to measure a range of variables, and not rely on one to act as a measure of ecosystem fitness.

A more detailed examination of macroinvertebrates, with identification to species level, may provide alternative results to this investigation. This could be for all family groups, or for a single group acting as an indicator for all macroinvertebrates. Water beetles may be a beneficial group to use as they are thought to act as a surrogate group for overall macroinvertebrate diversity (Gioria, 2010). The use of a single order as a predictive model may however lead to inaccurate data, being that the impact of *C. helmsii* on individual macroinvertebrate orders is unknown. The adoption of one single order as a predictor for a whole site assemblage would therefore be largely assumptive, and would require *a priori* investigations into suitable species to use as a representative for macroinvertebrates overall.

Site access was more restricted for macroinvertebrate surveys than for macrophyte surveys. This would need to be addressed for future studies. Retaining good relations with organisation was seen as imperative to carrying the surveys. To accomplish this, the loss of macroinvertebrate sampling and subsequent data for analysis was accepted to ensure access was available for the other study areas.

Conclusion

From the data that was collected and analysed, no measurable impact of *C. helmsii* on macroinvertebrates was found, with *C. helmsii* not having a detrimental effect on macroinvertebrate diversity. Further studies would be appropriate as a monitoring aid, to ascertain whether these results change over time. This may require investigation outside of the county, due to the limits on the number of sites with these attributes being found in Kent.

Further studies could be carried out on individual macroinvertebrate taxa, in an attempt to find a predictive grouping of species, such as the water beetle method (Gioria, 2010). This would facilitate faster and more efficient evaluation of sites, as the results have suggested no predictive correlative functions between macrophyte and macroinvertebrate assemblages studied. Delimiting the surveying methods to different profiles within the vegetation may also provide new insights. It has been shown that *I. glandulifera* supports different species and causes varying effects dependant on whether sampling is above ground or below ground (Tanner *et al.*, 2013). The effects of *C. helmsii* on macroinvertebrates may have similar results, and so a more detailed study that looks at different methods of sample collection, which included terrestrial species, would likely give a more detailed analysis.

No impact of invasion was found when using a community scoring index devised from the data collected. The use of the BMWP pollution derived index that is normally used to measure water quality, was also found to have no significant difference when used to compare species found on invaded and control sites. It was felt important to investigate both indices, as scoring macroinvertebrates dependant on pollution matrixes was not felt to be an accurate way of portraying invasion effects. Further development of this method, which uses the species present on control sites as a measure of a natural, uninvaded habitat could be continued. Impacts on these waterbodies, including nutrient enrichment and species disturbances (possibly by other invasive species) would need to be addressed in order to make it a more accurate method.

This study did not make a measure of abundance, due to the scoring systems used and the issues surrounding collecting, preserving and identifying all specimens collected. Species diversity has been shown to act as representative group for the ecology of wetland ecosystems (Awal and Svozil, 2010). It has also been used as a method identifying both biotic and abiotic impact in previous waterbody studies (Lepori *et al*., 2005; Petrin *et al*., 2007; Stranko *et al*., 2012; Keitzer and Goforth, 2013) as well as being used to calculate PSYM and BMWP metrics. Including abundance measures may show how invasion has caused changes in total numbers of species, and so allow for calculations as to which species have been impacted the most. This would be a key area of investigation for future studies, and would enable a more comprehensive evaluation of invasion effects on macroinvertebrates to be compiled.

Chapter 6 – The Effects of *Crassula helmsii* on Soil Seed Banks

Introduction

The growth of a non-native macrophyte species may have potential implications for the seed bank of a habitat. The seed bank is used as an indicator of the seed reserves within the top soil and litter layer (Roberts, 1981). The quantification of a habitat's seed bank, the overall composition of seeds within the topsoil layer, may provide important information on a habitat resilience to invasion (Mason *et al.*, 2007), as well as a measure of its genetic diversity (Fennell *et al.*, 2014). The quantification of a seed bank acts as a predictive tool for possible future species assemblages (Gioria *et al.*, 2012). Sternberg *et al.* (2003) suggested that that a quantified seed bank could be used to help make more informed habitat management decisions. Knowledge of a seed bank would aid in choosing between eradication or containment of an invasive macrophyte species (Fletcher *et al.*, 2015). The ability to predict long term changes to a habitat due to invasion allows for resources to be better allocated in managing invasive species, which are often limited and otherwise poorly allocated (Merchant *et al.*, 2011). Data-driven decision making also has implications for restoration of sites post management, with the necessity for the manipulation of soil, litter layers or both to be a prerequisite for successful re-colonisation of native species (Chenot *et al.*, 2014). The importance of seed bank management and artificial manipulation by restricting fruiting bodies is seen as an integral part of an integrated management strategy of tackling non-native species (Richardson and Kluge, 2008).

A limited number of studies have been carried out on the relationship between invasion by non-native macrophytes and their effects on the seed banks of a habitat (Vila and Gimeno, 2007; Fisher *et al.*, 2009; Kundel *et al.*, 2014.) Gioria *et al.* (2012) summarised 18 separate studies on the effects of plant invasions on seed banks. In 15 of these studies, a statistically significant change in species composition was found, with 13 of the studies showing effects on the seed bank. A variety of reasons for these changes are discussed (Gioria *et al.*, 2012).

The evidence from previous studies on the impact of non-native macrophyte species on seed banks is inconclusive. Non-native species were thought to be responsible for the decrease in diversity of native species through modifications of the seed bank in a study of *Banksia* woodland in Australia (Fisher *et al.*, 2009). A reduction in native species over time was shown by Fisher *et al*. (2009) of *Ehrharta calycina* (Purple Veldtgrass) and P*elargonium capitatum*

(Rose Geranium), both South African perennial species that have invaded *Bansksia* woodland in Australia. Changes to habitats which included riparian corridors, wet meadows and grasslands were attributed to the effects of invasive species on seed banks (Vosse *et al.*, 2008). A study of the clonal grass *Stenotaphrum secundatum* found a decrease in seed bank species diversity due to invasion, as well as a significant difference between the seed bank and standing biomass (Gooden and French, 2014). C*hrysanthemoides monilifera ssp. rotunda* (Bitou Bush) were found to have significantly higher native tree species seeds within the seed banks when compared to only lightly invaded sites (Mason *et al.*, 2007).

Research has shown species specific results, with differences occurring over time that were either detrimental to the seed bank diversity, or showed no effect (Gioria *et al.*, 2012). A previous study on the effects of *C. helmsii* found that germination of native species was affected, with a decrease in germination success from 46.6% to 24.7% when *C. helmsii* was present (Langdon *et al.*, 2004). However, a change in the seed bank was not thought to be the cause, as further studies indicated no significant change in diversity. An average of 25.8 species was found on invaded sites, compared with 24.8 species on control sites. A study of the invasive species *Solidago gigantea* (Giant Goldenrod) and *Solidago canadensis* (Canadian Goldenrod) found no effect of invasion on seed bank size, species richness or species diversity (Kundel *et al.*, 2014). Invasion was thought to account for only 10% in species variation, with site specific 'background' variation accounting for 90% of the variation. The seed banks of Menorca were shown not to be affected by invasion by geophyte species, with uninvaded areas not having a significantly larger or more diverse seed bank than areas invaded by the geophytes species studied (Vila and Gimeno, 2007).

A range of factors are suggested for why the establishment of non-native species could lead to decreases in seed bank diversity. Germination suppression is when a macrophyte species prevents the establishment of other species. Suppression occurs through the limiting and removal of resources from a system, therefore favouring the invasive species and reducing the likelihood of the establishment of competing natives (Langdon, 2004). The interception of 'seed rain' is another possibility. Interception is the prevention of seeds reaching suitable substrates for successful germination, due to capture within the canopy layers (Rogers and Hartemink, 2000; Gioria and Osbourne, 2010). Interception compounds the decrease in seed rain, through a reduction in seeds being produced.

The establishment of a dense litter layer can cause an increase in the interception of seed rain, with the propagules not able to reach the ground layer and germinate (Rogers and Hartemink,

2000). Dense litter acts as a preventative measure for germination if the seeds or plant fragments are not able to penetrate the mulched layer, with a decrease in light and a change in water availability acting to decrease the likelihood of growth (Rogers and Hartemink, 2000).

A change in natural disturbance regimes, such as an alteration to flooding events (Casanova and Brock, 2000) can lead to a decrease in native species establishment. The duration of propagule submergence was shown to change germination success, with differing levels of anoxia leading to changes in successful establishment (Casanova and Brock, 2000). Riparian habitats are vulnerable to changes in water regulation through factors such as management and climate change (Vosse *et al.*, 2008). Changes in water level can lead to the exposure of bare ground on the drawdown zone between winter and summer levels for different amounts of time, thus causing disturbance to native species establishment. These changes have been shown to occur for both permanent and temporary ponds, which dry out or remain wet for varying lengths of time (Casanova and Brock, 2000).

Direct competition between natives and invasives during the germination stage of reproduction can account for changes in species, and therefore decreased recruitment. Invasives are often able to spread reproductive propagules over a wide area, which remain dormant until favourable conditions exist (Fisher *et al.*, 2009). A study of germination success over time has shown that invasives, which showed a 30% germination success in their first week, were better able to colonise an area than comparable native species, which showed only 4% germination success in their first week (Fisher *et al.*, 2009).

Aims

The aim of the seed bank investigation was to determine whether any measurable impact of invasion by *C. helmsii* on seed bank diversity and total number of seeds could be found. The act of management to tackle invasion by *C. helmsii* was also investigated, to better understand the impacts that artificial control practices have on the seed banks of the sites. A comparison between standing macrophyte coverage and below ground seed banks was also carried out, to determine whether a sites seed bank could be predicted by measuring only above ground biomass. The long term effects of invasion were also studied, to determine whether seed banks were affected by the length of time that *C. helmsii* had been present.

Methods

The study of seed banks of UK native species are lacking, with minimal information existing on protocols for surveying and identification of UK native species (Pennick, 2012). Protocols were therefore adapted from information taken from agricultural manuals (Martin and Barkley, 2000; NIAB, 2004) and soil surveying manuals (Roberts, 1981). Soil samples containing seeds were collected in accordance with the method stated in Chapter 3, Survey Methodology. These were collected from sampling locations across Kent and East Sussex, on both invaded and uninvaded control sites. A range of habitat and waterbody types were selected so as to provide a wide variety of seed bank sources. Seeds were separated from the soil using a combination of sieving, chemical flotation and air flow separation. A more detailed description of these methods can be found in Chapter 3.

Once dried, the seeds were identified using a combination of hand lens, lower power microscope and visual identification.

Figure 6.1. Seed samples after air-flow separation, prior to hand sorting. The samples were sorted by hand into individual groupings to assess seed bank diversity.

The seeds were sorted in accordance to a key constructed to enable classification by size, shape and colour. This key was developed during the process of sorting, and added to continuously as new seed specimens were isolated and identified.

Table 6.1. The seed key used to classify the seeds identified after extraction. The key was developed during sorting as new seed types were found.

Seed Code	**Seed Description**		
A	BLACK	ARROW	LARGE
B	BROWN	FLAT	
C	BROWN	ROUND	
D	BROWN	IRREGULAR	
E	PALE	ELONGATED	
F	BROWN	ELONGATED	
GA	BLACK	ROUND	SMALL
GB	BLACK	ROUND	SMALL
H	BROWN AND PALE	ELONGATED	
I	BROWN	ALMOND	
JA	BROWN	LARGE	
JB	BROWN	LARGE	
K	PALE	ARROW	
LA	PALE	OBLONG	SMALL
LB	PALE	OBLONG	SMALL
M	PALE	WALNUT	
N	BLACK	OBLONG	LARGE
O	BLACK	IRREGULAR	
P	PALE	IRREGULAR	
Q	BLACK	ELONGATED	SMALL
R	BROWN	HONEYCOMB	
S	PALE	ROUND	SMALL
T	BLACK	ELONGATED	
U	BROWN	ROUND	SMALL
V	PALE	DOUGHNUT	
WA	BLACK	ROUND	LARGE
WB	BLACK	ROUND	LARGE
X	PLAE	ROUND	
Y	PALE AND BROWN	ROUND	
Z	BLACK	FLAT	IRREGULAR

Seed viability was verified visually by looking for fragmentation of the seed coat and by applying pressure to enable identification of solid organic particles (stone particles) that had remained after separation techniques, which were disregarded using this manner.

The seeds were manually sorted after identification through the use of the key (Tab. 6.1), into spotting wells. This allowed for both diversity and abundance of seeds to be measured. On completion of sorting of the samples, the seeds were placed into small polythene bags, with the subsequent classification of seed diversity (letter/number combination) and seed abundance (number) labelled onto the bag.

Some seed classifications were recognised as having more than one type of seeds present after keying out. These groupings were subsequently adjusted to allow for a true diversity score to be constructed, by factoring in additional groupings if more than 3 seeds were found within these pools. Species richness of the seed bank in invaded versus uninvaded sites (Tab. 6.2) was compared using Mann-Whitney U tests. The seed bank species richness for invaded sites with managed and unmanaged status (Tab. 6.3) was also compared using a Mann-Whitney U test.

Table 6.2. Seed sample numbers from direct comparison between *C. helmsii* invaded sites and control sites.

Site Name	Samples from *C. helmsii* Sites	Samples from Control Sites
Oare	5	2
Gunpowder Works	0	3
Orlestone	0	2
Hothfield	2	4
Shorne Woods	4	0
Bough Beech	5	1
Sevenoaks	5	1
Perry Woods	2	0
Rye Street	5	2
Rye Harbour	5	0
Stodmarsh	5	2
Beacon Woods	2	0
Blean Woods	2	0
Eastry	2	0
Fowlmead CP	0	3
Dungeness	4	1
Romney Marsh	2	0
Total	**50**	**21**

Table 6.3. Seed sample numbers from management comparisons on invaded sites.

	Site Designation	Samples from *C. helmsii* Sites
Management Type	**Unmanaged/Target Managed**	18
	Active Management	32

To enable a comparison of seed data and macrophyte data, macrophyte species number and conservation scores for each site were used for sites where seed data was available. The data

used from the seed analysis was the total number of seeds and diversity scores for each site. Analysis was by linear correlation.

To assess the change to the seed bank over time, each site where *C. helmsii* was present was dated in accordance to the earliest available record. Dating information was taken from information gained from Kent Biological Records Centre (KMBRC, 2012). This ranged from dates of 1990 for Blean Woods, to the most recent dates of 2012 for the Rye Street Reserve. Dating information was correlated to both seed diversity and total seeds for each site.

Results

Comparison between *C. helmsii* invaded sites and control sites

There was no significant difference between invaded and uninvaded sites for diversity (Mann-Whitney U test; P=0.5235) and total numbers of seeds (Mann-Whitney U test; P=0.7691) (Tab.6.4).

Comparison between management activities

There was a significant difference between sites that had been managed and sites that had not been managed to control *C. helmsii* invasion (Mann-Whitney test, P=0.0391) (Tab. 6.4). Box plot analysis showed that seed diversity was lower on invaded sites that were managed compared to unmanaged invaded sites (Fig. 6.2).

Table 6.4. Results of the Mann-Whitney statistical analysis using seed data. The management comparison for seed bank diversity was statistically significant (0.0391).

Comparison Test		**P-Value**
***C. helmsii* vs. Control Sites**	**Diversity**	0.5325
	Total No. Seeds	0.7961
Management	**Diversity**	0.0391
	Total No. Seeds	0.0510

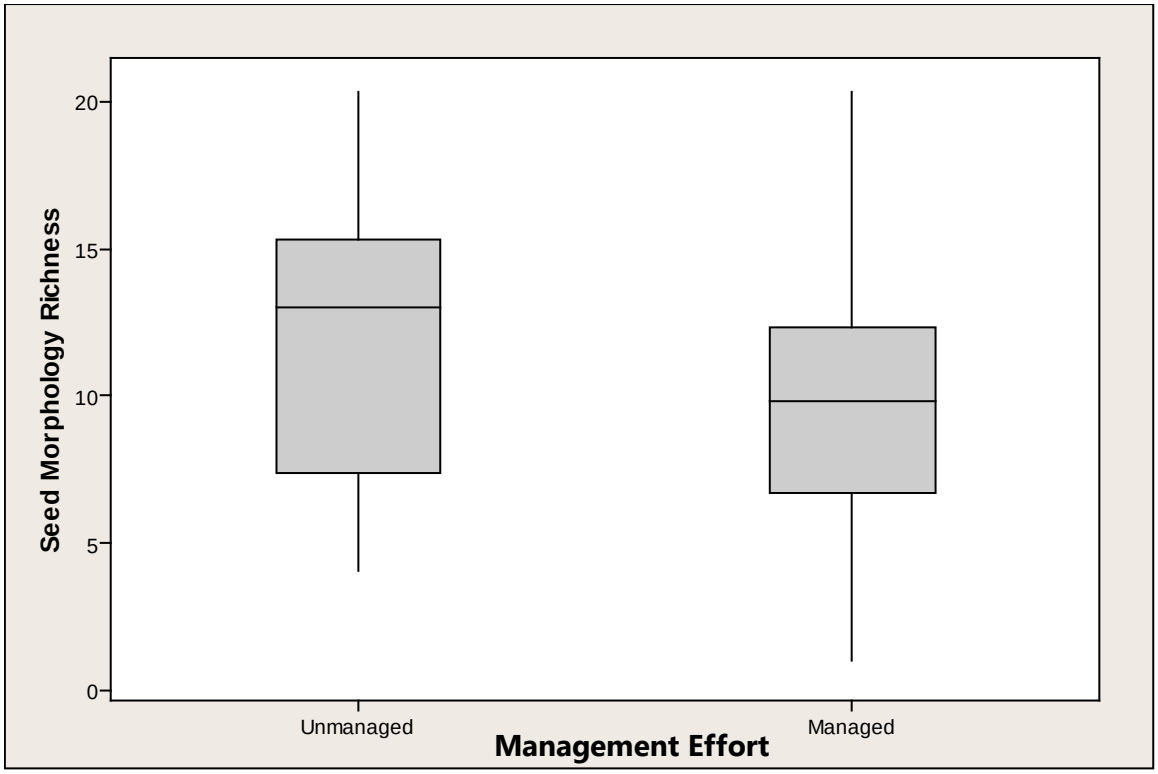

Figure 6.2. Boxplot comparison of seed morphology richness between managed and unmanaged sites supporting *C. helmsii*.

Correlation between seed banks and macrophyte assemblages

To assess whether macrophyte assemblages acted as a predictive measure of seed bank characteristics, the data between the two groups was compared by a correlation of the two data sets. Macrophyte diversity and average site rarity scores were compared with seed bank diversity and abundance individually.

Of the 12 separate tests that were carried out, only 1 returned a statistically significant response (Tab. 6.5). This was the comparison of 1982 macrophyte conservation scores plotted against the total number of seeds (Pearson's correlation = 0.282) (Fig. 6.3).

Table 6.5. Results of the correlation between plant and seed data. Only one value was found to be significant (highlighted).

Plant Data Type	Seed Data Type	Pearson's Correlation Value	P-Value
No. of Species	Diversity	0.131	0.365
ATLAS Average Score	Diversity	0.104	0.472
BSBI Average Score	Diversity	0.101	0.484
PSYM Average Score	Diversity	0.146	0.311
1982 Average Score	Diversity	0.104	0.472
2010 Average Score	Diversity	0.081	0.574
No. of Species	Total No. Seeds	0.181	0.208
ATLAS Average Score	Total No. Seeds	0.123	0.394
BSBI Average Score	Total No. Seeds	0.11	0.446
PSYM Average Score	Total No. Seeds	0.043	0.769
1982 Average Score	Total No. Seeds	0.282	0.047
2010 Average Score	Total No. Seeds	0.265	0.063

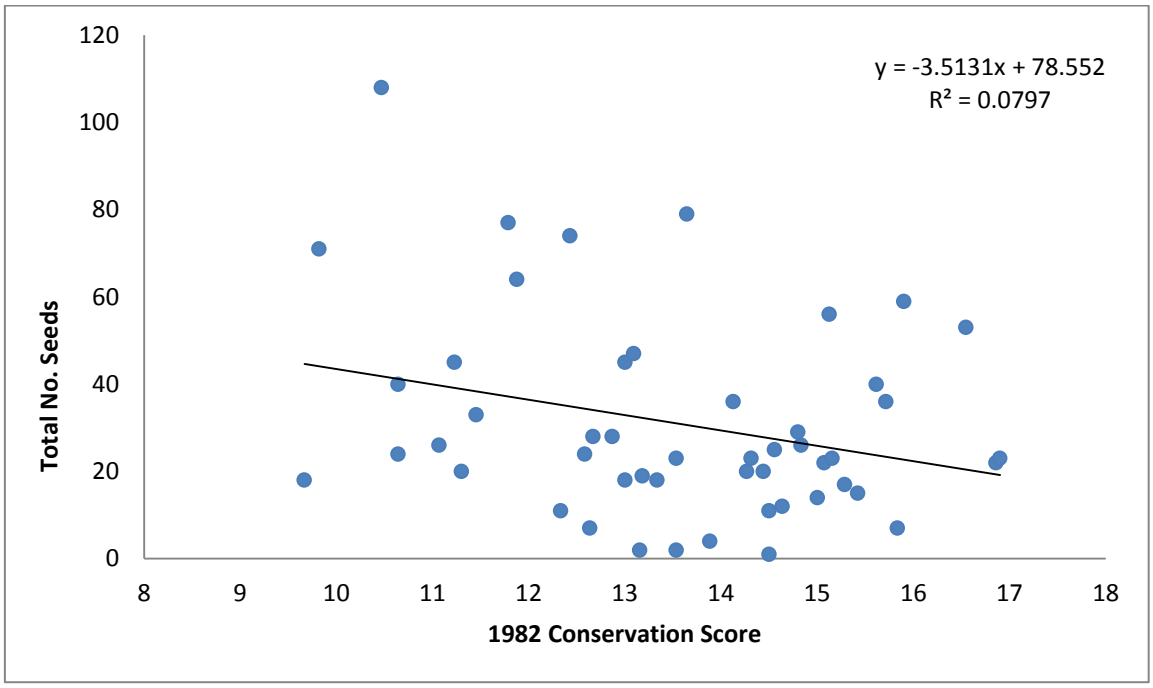

Figure 6.3. Comparison of 1982 macrophyte data and seed data, showing a weak correlation between the two data sets. This was the only comparison that was statistically significant, but showed a weak correlation with little predictive power.

Comparison of seed bank measurements against duration of presence by *C. helmsii*

The comparison of seed bank diversity and seed total number against year of discovery of *C. helmsii* found no statistically significant relationships for diversity (Correlation test; P=0.198) (Fig. 6.4) or for the total number of seeds (Correlation test; P=0.147) (Fig. 6.5).

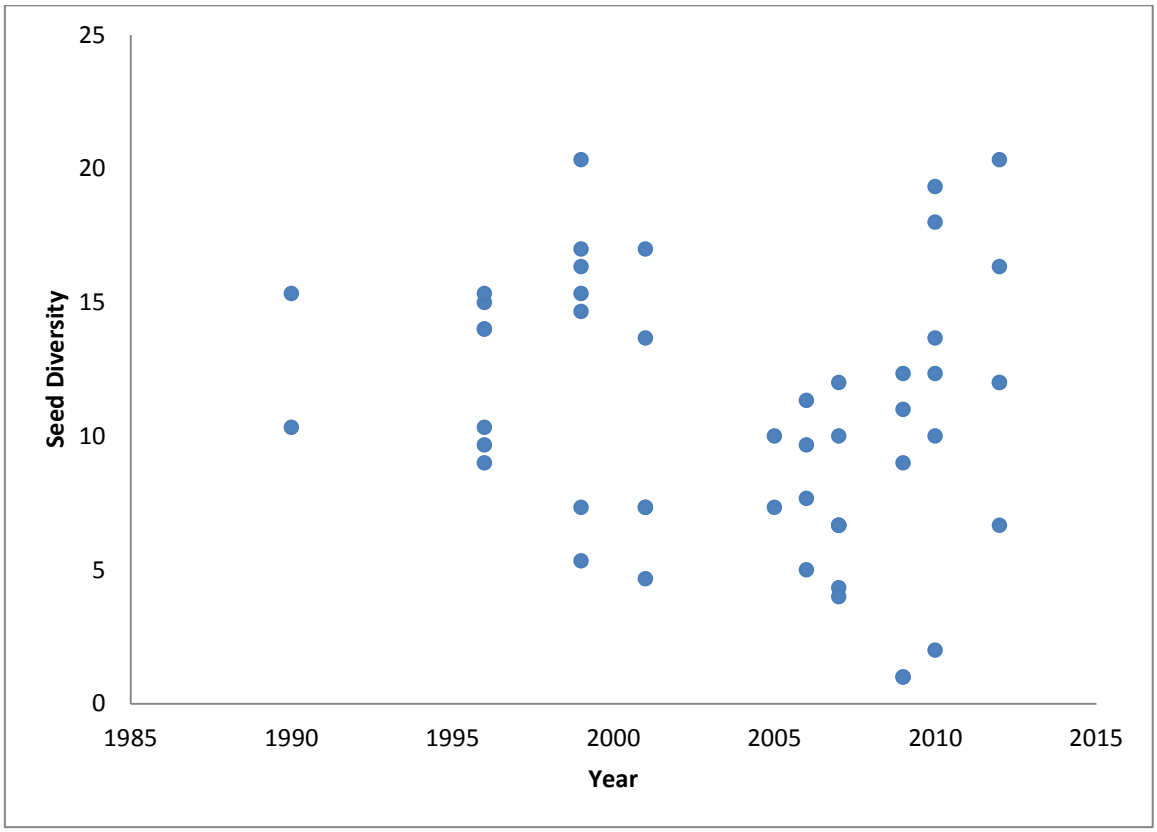

Figure 6.4. Comparison of seed bank diversity against year of discovery of *C. helmsii*. No correlation was found between the datasets.

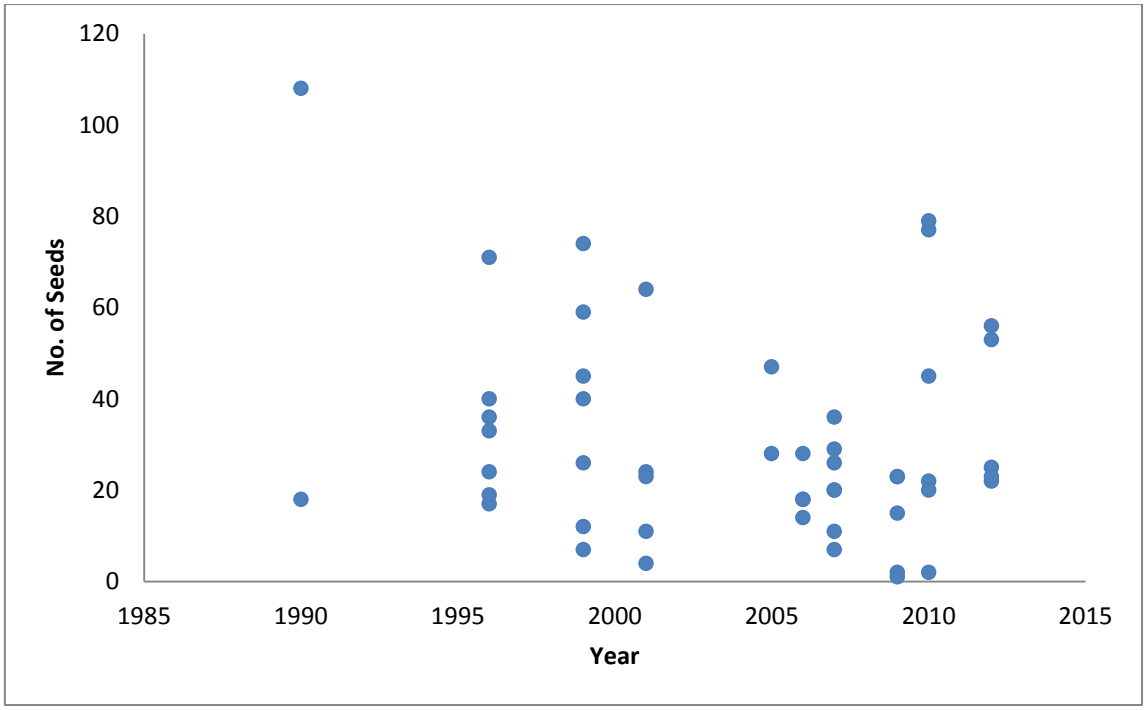

Figure 6.5. Comparison of total number of seeds diversity against year of discovery of *C. helmsii*. No correlation was found between the datasets.

Discussion

Comparison between *C. helmsii* invaded sites and control sites

Invasion by *C. helmsii* did not cause a decrease in seed bank diversity or total number of seeds in this study. The comparison between invaded and uninvaded control sites showed no statistical difference in diversity. It can therefore be seen that this invasive does not appear to be detrimental to a sites seed bank post invasion, at this point in the invasion sequence.

These results support the only other published study on the seed banks of sites invaded by *C. helmsii*. Langdon *et al*. (2004) had previously shown that invasion caused no change in seed bank density (the same measure as diversity in this study). It is also supported by a study on the effects of invasion by *Solidago spp.*, which found no effects on the seed bank when compared with uninvaded control sites (Kundel *et al.,* 2014). A study of invasion by annual geophyte species on the native species in field sites in Menorca also found no difference between invaded and uninvaded control sites for species richness and seed bank size (Vila and Gimeno, 2007).

The effects of seed rain interception by *C. helmsii* would appear not to be having an adverse effect upon seed bank measurements, even though *C. helmsii* is able to create dense stands on some sites. The decrease in seed rain due to interception was found to be a contributory factor to differences in seed bank density in a study of *Piper aduncum* (Matico) in Papau New Guinea (Rogers and Hartemink, 2000). In their study, interception of native species' seeds by the tree canopy was thought to account for a decreased seed bank within the forest sites, in comparison to the fallow sites. The difference in height of the canopy may however account for the differences between trees and *C. helmsii* as an intercepting layer. Tree species decrease the chance of seed species from reaching the soil layer, due to the height of the canopy acting as a deflecting structure (Rogers and Hartemink, 2000). As *C. helmsii* is a low growing macrophyte, whilst it may stop the initial seed delivery from reaching the soil layer immediately, it is likely that seed percolation would still occur due to the spaces that remain between the leaves and stems. Therefore whilst seeds are slowed from reaching the soil layer from the native seed rain, it is unlikely to stop them from reaching the soil layer and eventually becoming incorporated into the seed bank.

Another factor that may explain why seed bank diversity has not decreased under *C. helmsii* invasion is due to the current understanding of the germination of *C. helmsii* seeds being unclear (Dawson and Warman, 1987; Dawson, 1994; Denys *et al.*, 2014). Invasive species that do produce viable seeds, which subsequently incorporate into the seed bank, alter the seed bank before the invasive species is present above ground (Fisher *et al.*, 2009). Therefore integration into the native seed bank may not be occurring prior to invasion. Lack of displacement by the seeds of *C. helmsii* may be challenged if it is found that germination success can be achieved, which has only been found when inoculating soils with the flower head, rather than the isolated seed (Denys *et al.*, 2014).

Comparison between management activities

Management of *C. helmsii* appeared to significantly decrease seed bank diversity. Active management is non-target specific, with the potential to impoverish native species and disturb the topsoil layer. Increases in unnatural disturbance patterns have been shown to favour invasive species, and be detrimental to native species (Burke and Grime, 1996). Germination success of invasives was also shown to be greater than comparable native species (Fisher *et al.*, 2009). Though the effectiveness of *C. helmsii* seeds is unknown, its ability to reproduce

asexually by fragmentation is well recognised (Dawson and Warman, 1987; Kane *et al.*, 1993; Hussner, 2009). On sites where *C. helmsii* is managed in a way that leaves bare ground, successful colonisation from neighbouring growth of *C. helmsii* is likely to occur. Though no chemical fertiliser is intentionally applied at the management stage, the removal of native species could leave an unused pool of nutrient available, and thus remove its likelihood of being a limiting factor to growth. Such management provides *C. helmsii* with both the increases in disturbance and increases in nutrient, which in combination has been shown to be advantageous for non-native establishment (Burke and Grime, 1996). High impact control in this manner is likely to also remove the competition from native species through the seed bank, either with the complete removal of the seed store by topsoil stripping (Dawson and Warman, 1987; Leach and Dawson, 2000), or a reduction in seed rain due to the loss of native species releasing seeds to the seed bank (Rogers and Hartemink, 2000). These results are supported by a study of coastal dune seed banks, where the invasive species C*hrysanthemoides monilifera ssp. rotunda* (Bitou Bush) was found to have significantly less native tree species seeds in managed habitats compared to only slightly invaded sites (Mason *et al.*, 2007).

Removal of the native macrophyte assemblages may also leave a site prone to invasion from satellite communities. Poor management decisions have been shown to increase the success of invasives in areas where they were not previously present, but were within close proximity to an established invaded site (Fisher *et al*., 2009). The state of the seed bank after invasion has been found to be an important factor in restoring native species (Vosse *et al*., 2008). Therefore any management activity that causes degradation to a site for short term gains (removal of *C. helmsii* plant material) is likely to have detrimental long term impacts, by both leaving a site vulnerable to re-colonisation and removing the ability for native species to recoloinse from the seed bank. It has been shown that management by removal of above ground biomass is effective only when disturbance to the site is low and the invader has delivered a low number of propagules (Firn *et al*., 2008). If disturbance is unavoidable, then focus should change towards removing propagules rather than plant biomass (Firn *et al*., 2008). For *C. helmsii* management, this could mean acting to prevent further spread by the prevention of propagule spread, rather than targeting the plant itself. Studies on *Carpobrotus spp.* (Iceplant) on the Mediterranean Islands found that litter removal (which contained a large seed stock of *Carpobrotus* seeds) was required for successful control of the plant (Chenot *et al*., 2014). This is further supported by a study of *Acacia spp*. in South Africa, where it was found that targeting the removal of buds, flowers and seed pods was more beneficial to

reducing the number of *Acacia spp.* seeds within the seed bank than controlling the whole plant (Richardson and Kluge, 2008). When considering *C. helmsii*, the asexual reproduction techniques that it utilises through fragmentation (Dawson and Warman, 1987) means that the whole of the above ground biomass is a source of reproductive propagules. Therefore targeted control of a particular part of the plant is not possible, and may further explain how the plant is able to rapidly colonise a site once present. It also provides an explanation of how disturbance through management can lead to an enhancement of its ability to colonise.

A further management strategy that could cause this level of disturbance is the use of grazing livestock. Many of the sites surveyed were or had previously been managed through grazing. The use of this method of management may have benefits, such as scrub control, but the effects towards the seed bank have been shown to be negative under certain conditions (Sternberg *et al.*, 2003). Grazing was shown to enhance the growth of *Cytisus scoparius* (Common Broom) in a trial of management techniques in Australia (Sheppard *et al*., 2002). The impact of grazing may also lead to artificial disturbance patterns, which have been shown to promote the establishment of invasives (Burke and Grime, 1996; Firn *et al*., 2008). The data has shown that management is reducing the ability for native seed banks to recolonise naturally, and may provide an explanation as to why *C. helmsii* has remained an unmanageable species to date.

Correlation between seed bank and macrophyte assemblages on *C. helmsii* invaded sites

Analysis of seed bank abundance and diversity with macrophyte species number and average rarity score found little relationship between the two sets of data. Table 6.4 shows one statistically significant value (p=0.047) between the total number of seeds and the Kent 1982 scoring metrics, but a low correlation value. It can therefore be concluded that it is unlikely that above ground macrophyte presence on *C. helmsii* invaded sites acts as a predictive indicator of seed bank assemblages. It therefore should not be used as a method of estimating the seed bank, and direct sampling and identification remain integral to analysing the seed bank. The disparity between the seed bank and above ground macrophyte presence is supported by another wetland study. An investigation of coastal seed banks invaded by the grass *Stenotaphrum secundatum* found no statistically significant link between seed banks and macrophyte coverage, on either invaded or control sites (Gooden and French, 2014). This was thought to be primarily due to a reduction in primary recruitment from the seed bank rather

than seed delivery to the soil (Gooden and French, 2014). A study of above ground biomass and soil stored seeds in South African shrub-lands also found a poor correlation between the two (Holmes and Cowling, 1997).

The reason for the low correlation value between macrophyte and seed data could be due to a disturbance in seed rain. Though it has been shown in this study that the number of macrophyte species does not change significantly post invasion, the location of these seed bearing plants may be affected by displacement by *C. helmsii*. Therefore the seed rain to a specific point (the location that was sampled) may have been altered. Macrophyte abundance may not be representative of a single locations seed bank sample. An increased number of samples from each site would give a greater level of confidence in the representation of the seed data representing the status of the invaded site. Rogers and Hartemink (2000) described how the seed rain plays a key role in determining the seed bank of invaded sites, with interception and changes to canopy structure resulting in changes to the underlying seed bank. The accumulated native seeds within the seed bank may remain present after invasion has caused noticeable changes to macrophyte distribution above ground (Sternberg *et al.*, 2003). Therefore, the seeds that are identified by sampling and separation are unlikely to portray what is above ground, acting more as an historical record than a reflection of the current assemblages (Thompson *et al.*, 1997; Gioria *et al.*, 2012). The soil and seed bank provide evidence of what was present on a site previously, but may not accurately reflect what is currently growing there at the time of sampling.

Comparison of seed bank measurements against duration of presence by *C. helmsii*

No correlation could be found between the length of time of invasion and the impact upon seed bank composition. From this data therefore, relationships of change over time between seed bank diversity and density cannot be found. This may be due to the length of time that *C. helmsii* has been present on the sites studied not being long enough to cause changes. It is known that the residence time of seeds within the seed bank can differ depending on the species present. These have been classified as transient species (<1year), short-term persistent species (1-5years) and persistent (>5years) (Thompson *et al.*, 1997). As some sites are recorded as being invaded only 1-2 years before the surveys were carried out, it may be that the true effects of invasion over time requires a greater period of *C. helmsii* establishment.

This was stated as a possible reason for no effects being found in a previous study of seed banks on sites invaded by *C. helmsii* (Langdon, 2004).

Different invasive species are known to have varying effects upon native species over time, with some able to cause changes over a short time, whilst others had less impact. *Fallopia japonica* (Japanese Knotweed) was shown to alter species richness and abundance of the seed bank over a short time period (Gioria and Osborne, 2010). This was not observed for another two invasive species studied – *Heracleum mantegazzianum* (Giant Hogweed) and *Gunnera tinctoria* (Chilean Rhubarb). These effects are likely to involve interactions such as interception and suppression through litter, which will vary dependant on the species' morphology and decomposition rates. As a comprehensive dataset for the effects of invasive species towards natives over time is lacking (Gioria *et al*., 2012), being able to compare these short term effects with other species is difficult and likely to be inaccurate. Further investigations may include studying the seed banks of sites that have known *C. helmsii* presence over a longer time period. This would enable the data to be extended over a longer period of invasion, and therefore allow for more accurate analysis to be carried out. Difficulties surround this however. Site management histories may not be known, which might occur if the site has been managed by different organisations. This is likely for nature reserves that have been managed for wildlife over long time periods. Poor record keeping of management activity may also hinder accurate data collection, as well as changes in management type.

Data accuracy would be integral to carrying out this analysis. The initial invasion date was taken from data received from Kent Biological Records. These records are maintained by this organisation, but are dependent upon the submitted records being accurate. Whilst attempts were made to verify this data with land managers and site owners, due to changes in staffing and site ownership it was difficult to verify the dates accurately. As the plant is difficult to identify against similar natives such as the *Callitriche spp.* (Water Starworts), *C. helmsii* may have been present on a site for longer than was noticed by the land managers. Therefore reports of its initial presence may be erroneous, and subsequently lead to data not being accurate.

The continued monitoring of the seed bank of invaded stands should continue, to ascertain whether the prolonged invasion leads to a deterioration. A study of *Acacia longifolia* (Golden Wattle) in Portugal showed that species richness, seedling density and seed diversity were all significantly higher on control and recently invaded sites compared to those invaded over a longer period (Marchant *et al.*, 2011). It also illustrated that short term invasion was similar to

no invasion when examining the effects upon the seed bank (Marchant *et al.*, 2011). When related to *C. helmsii* invasion, further sampling effort would allow for a more comprehensive conclusion to be made about how time scales affect seed banks, allowing for more informed management decisions to be made. This may result in the eventual use of native seed stocks being applied artificially for re-colonisation of sites post management, in circumstances where removal of the invasive is no longer enough to allow succession back to pre-invasion status.

Conclusion

The data and subsequent analysis has shown that invasion by *C. helmsii* does not cause a change in seed bank diversity or abundance over time. A possible detrimental effect of active management strategies to remove *C. helmsii* by non-target control has been shown. The suggested implications of this are effects on the re-establishment of native species post management. This should highlight to land managers and organisations the possible impact of these management strategies for long term recovery of a site, with the reduced likelihood of regeneration of native macrophyte species due to a reduction in the seed bank.

An attempt to use macrophyte cover as a predictive measure of seed bank diversity and abundance has been shown to be unrealistic and inaccurate. This may be due to issues including artificial manipulation through management, changes in seed rain and seed rain interception by the invasive.

No correlation could be found for a decrease in seed bank measures over time. The length of time of invasion by *C. helmsii* could be further explored, as an increased duration of *C. helmsii* presence may indicate changes to this relationship. This could be addressed by a continued monitoring effort, in an attempt to discover whether this is altered with the availability of more data over a longer study period.

Chapter 7 –The Effects of *Crassula helmsii* on Water Chemistry

Introduction

Human-mediated disturbance can lead to changes in habitat composition through the introduction and establishment of non-native species (Woods *et al*., 2003; Lake and Leishman, 2004; Leishman *et al*., 2005; Conesa and Jiminéz-Cárceles, 2007; Mason *et al*., 2007; Howden *et al*., 2013). Non-native invasive species are thought to be better suited to sudden changes to a habitat, which native species are thought to be less capable of adapting to rapidly (Dostal *et al*., 2013). A change to natural flooding regimes has been shown to lead to alterations in species composition and diversity (Daehler, 2003). In lentic systems, this could impact the refreshment rate of water to the waterbodies. Losses may also be due to evaporation, as is experienced by temporary ponds, with subsequent concentrating effects of compounds within the water (Birken and Cooper, 2006). Changes to the period of desiccation in temporary lentic systems could lead to the loss of native species, which may not have adaptations necessary for survival in these changing systems (Cassanova and Brock, 2000). Brewer (2010) has however shown that the impact of desiccation upon native and non-native species can vary, with no single rule existing for both species type. The consequence of these new and sudden changes could affect the water chemistry of a waterbody, as well as soil oxygen, nutrients and toxic substances (Casanova and Brock, 2000).

Human-mediated disturbance events of waterbodies can occur in a number of ways, not including the intentional use of disturbance as a management tool. Intensive farming practices and subsequent nutrient increases, caused by the overuse of fertilisers and pesticides, can lead to eutrophication (Howden *et al*., 2013). Proximity to agricultural land has been shown to elevate the nutrient levels of natural systems, through factors such as surface run-off and spray drift (Sandler *et al*., 2007). This increase in pollution events is thought to have caused an increase in pressure on UK ponds, which has led to species losses (Woods *et al*., 2003). Contrary to this is are the findings of Ehrenfeld (2008), who found that the number of invasive species decreased with an increase in the proportion of neighbouring commercial and industrial land, which was thought to be responsible for increased pollution inputs into the waterbodies of New Jersey, USA.

Invasive species have been shown to be able to utilise increases in resources more effectively when compared to native species (Lake *et al*., 2004). Nutrient increases have been shown to

lower macrophyte diversity in ponds (Williams *et al*., 2003; Wood *et al*., 2003). Conversely, low nutrient areas have been shown to prevent invasion by non-native species, with native species monopolising the lower amounts of nutrients available, thereby facilitating competitive exclusion of the non-native species (Stohlgren *et al*., 1998). Burke and Grime (1996) showed how increased nutrient levels promoted the establishment of non-native species, in conjunction with artificial disturbance. Nutrient addition was found to promote the growth of non-natives, but was not found to increase the biomass of native species (Leishman and Thomson, 2005).

The physiochemical ecology of *C. helmsii* across a range of habitats is unknown. The datasets that do exist are related to either the effects of management or tank trials, rather than the infield habitat status (Dawson and Warman, 1987; Hussner, 2009; Dean *et al*., 2013). Whilst it is recognised as a generalist species, quantified field measurements are lacking. The most complete previous study of the water chemistry of *C. helmsii* invaded sites was carried out by the Centre for Ecology and Hydrology, within the New Forest pond system (Brunet, 2002). This study concentrated on pH, conductivity, alkalinity, potassium and phosphorus measurements (Brunet, 2002). Further examination of the chemical variables, including the study of further water analysis of *C. helmsii* was deemed urgent, but no further evidence exists for this taking place. This limits any conclusion of whether *C. helmsii* shows a preference towards certain conditions, and therefore prevents any form of prediction as to which habitats may be suitable for invasion by this species.

C. helmsii has been shown to be a successful hyper-accumulator of copper (Küpper *et al*., 2009). It is capable of accumulating up to 9,000 ppm in plant dry weight, compared to 0.6ppm from a control group of macrophyte species (Küpper *et al*., 2009). Other aquatic invasive species have been found to have phytoremediation qualities. *Eichhornia crassipes* (Water Hyacinth) has been shown to increase the removal of phosphorus and nitrates from waste water, with increases of up to 36% when used as dry straw biomass (Chen *et al*., 2010). *Azolla carolinia* (Carolina Water Fern) has been shown to achieve a bio-concentration factor of up to 18.6 in its roots, when exposed to a range of heavy metals (Pandey, 2012). The ability for invasive species to exploit resources in this manner shows how they may have practical applications in the removal of nutrients and metals.

Aims

The aims of the water chemistry investigation were to collect and measure water samples taken from both invaded and uninvaded control sites. To investigate whether both individual and combined effects existed between the water chemistry data, a statistical investigation was conducted using binary logistic regression analysis. This was in an attempt to provide a possible predictive capability of a site's susceptibility to invasion.

Methods

Samples collected during field work were filtered and frozen for subsequent analysis (a full explanation of the procedure is shown in Chapter 3, Survey Methodology). pH was measured in the field using a Griffin Model 50 pH Meter. Measures of conductivity and TDS (total dissolved solids) were measured using a HM Digital Model Com-80 Digital Water Tester. Both meters were recalibrated on a weekly basis.

Samples for laboratory analysis were defrosted in ambient temperatures for 24 hours. After defrosting, samples were inspected for particulate matter to ensure filtration had been successful. To prevent any included sediments from entering the analytical equipment, samples were not mixed within the storage containers, with the higher fraction only being added to the specimen tubes. Total organic nitrogen (TON), ammonia and phosphate were analysed using a Burkard Series 2000 Automatic Chemical Analyser (Fig. 7.1). Tubes were loaded onto the disc in the order prescribed by the manufacturer's guidance, to include wash blanks and recalibration standards throughout. Any anomalies caused by concentration peaks or air bubble inclusion were re-sampled at the end of the run, within the same disk load to reduce variation.

- Total Organic Nitrogen (TON) concentrations were measured using the Automated Cadmium Reduction method.
- Ammonium concentrations were measured by the Automated Phenate method.
- Phosphate concentrations were measured by the automated Ascorbic Acid method. (APHA, 2005).

Figure 7.1. Burkard Series 2000 Auto analyser.

Samples were analysed for metals using flame photometry analysis on a Jenway PFP7 Flame Photometer (Fig. 7.2). Recalibration to a zero level was carried out at frequent intervals, to prevent the baseline from drifting and causing variation. No dilutions were necessary during the sampling effort, with all values remaining within the calibrated range of values of 0.0 mg l^{-1} to 1.0 mg l^{-1}.

Figure 7.2. Jenway PFP7 Flame Photometer.

Table 7.1. Water chemistry sample numbers from direct comparison between *C. helmsii* invaded sites and control sites. The site name column shows how the samples were segregated across the surveying locations.

Site Name	Samples from *C. helmsii* Sites	Samples from Control Sites
Oare	5	2
Gunpowder Works	0	3
Orlestone	0	2
Hothfield	2	4
Shorne Woods	5	0
Bough Beech	8	1
Bewl Water	5	0
Sevenoaks	5	1
Perry Woods	2	0
Rye Street	5	2
Rye Harbour	5	0
Stodmarsh	5	2
Beacon Woods	2	0
Blean Woods	3	0
Eastry	2	0
Fowlmead CP	0	3
Dungeness	5	1
Romney Marsh	2	0
Total	**59**	**23**

Results of the analysis were compared using the non-parametric Mann-Whitney test, with data organised into two groups of either invaded sites, or uninvaded control sites (Tab. 7.1).

Further analysis was carried using binary logistic regression in a stepwise manner. Each individual measurement parameter was included to give a single variable comparison. Binary regression enabled the use of a 0/1 coding system, with invaded sites designated a '1', and uninvaded sites a '0'.

Combined effects were analysed by creating a model, using the statistical package Minitab 17 to investigate 2 way effects. This was coded using the previously used binary system. The measurement parameters analysed for both single and combined effects were:-

- pH
- TDS (ppm)
- Conductivity (µS cm^{-1})
- Sodium (mg l^{-1})
- Potassium (mg l^{-1})
- Total Organic Nitrogen (mg l^{-1})
- Ammonia (mg l^{-1})
- Phosphate (mg l^{-1})

Results

Single Variable Analysis

The results of the direct comparison between sites invaded by *C. helmsii* and the uninvaded control sites using Mann Whitney analysis showed no statistical difference in water chemistry between the two site types (Tab. 7.2).

Table 7.2. Results of the water chemistry parameters compared using Mann-Whitney analysis.

Tested Variable	P-Value from Mann-Whitney Analysis
Sodium	0.8040
Potassium	0.4321
TON	0.2469
Ammonia	0.7881
Phosphate	0.0942
pH	0.8194
Conductivity	0.8029
TDS	0.1079

Binary Logistic Regression – Single Variable

The regression analysis, using a binary logistic model, returned a statistically significant result for total organic nitrogen (TON) (Binary logistic regression, P=0.005). The combined effect variables of TON and pH also showed a significant relationship (Binary logistic regression, P=0.009). The individual pH variable however was found not to be significant (Binary logistic regression P=0.225). All other tested variables were found not to be statistically significant.

Table 7.3. Results of Binary Logistic Regression that returned statistically significant results.

Variable Returned by Analysis	P-Value from Binary Logistic Regression
TON	0.005
pH	0.225
TON/pH Combined Effect	0.009

To assess the significant response returns, graphs were constructed of the single variable TON data, and the combined variables of TON and pH (Fig. 7.3).

The individual parameter of TON shows a decreasing probability of *C. helmsii* presence at higher levels of TON, and therefore a negative relationship between the two. A threshold level is apparent at approximately 4mg l^{-1}, whereby the probability of *C. helmsii* presence is at 0 (not present).

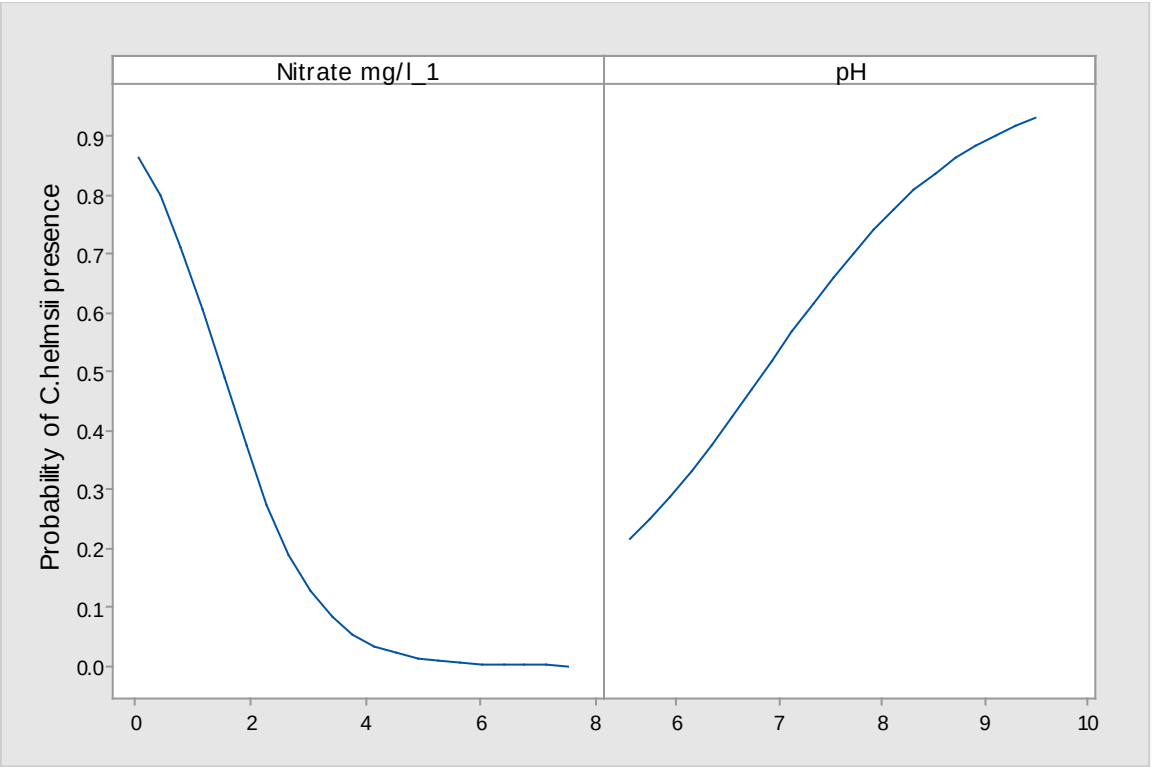

Figure 7.3. Single factor representation from binary logistic regression. TON (Nitrate) is measured in mg l^{-1}. pH showed no relationship as a single factor variable, but has been included above due to its relationship in the two factor analysis. (0=No *C. helmsii*, 1= *C. helmsii* present.)

Binary Logistic Regression – Two Variables

The statistically significant result returned by the regression analysis of the combined effects of TON and pH show that an increased TON measurement predicts a reduction in the probability of *C. helmsii* presence (Fig. 7.4). When the pH variable is included, this changes at approximately pH 8. At this pH level, TON is no longer acting as the only limiting factor towards *C. helmsii* presence.

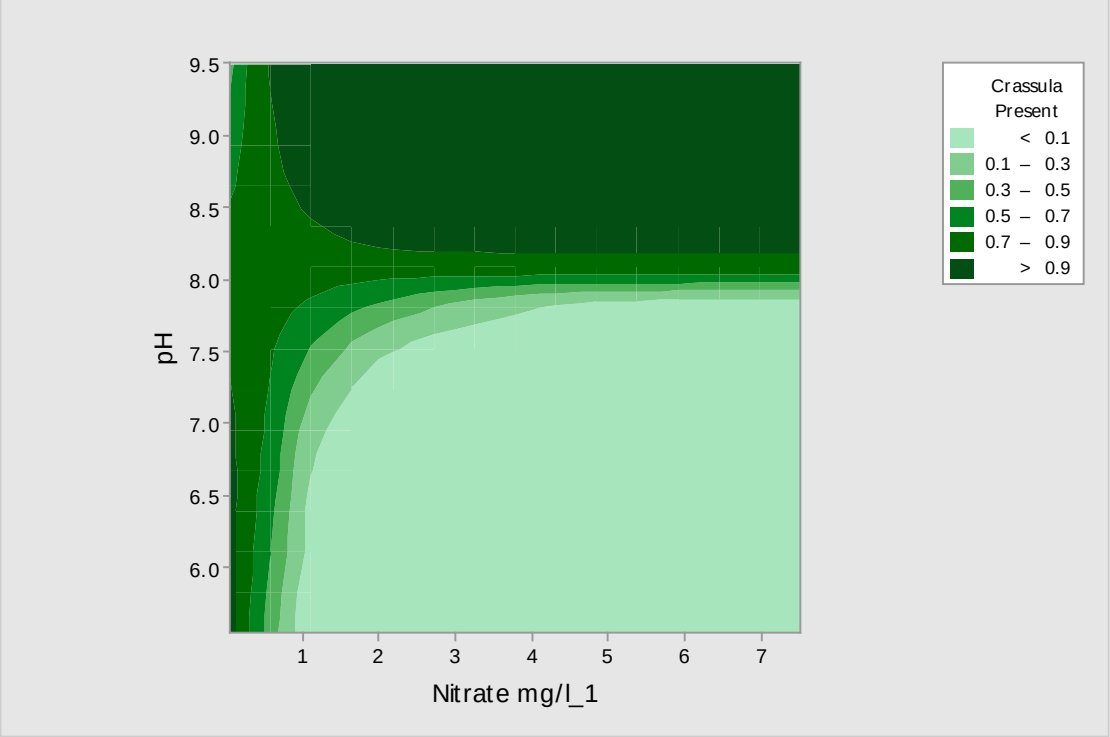

Figure 7.4. A plot of the combined effect variable of pH and TON (Nitrate). The higher value (dark green) represents an increased likelihood of the presence of *C. helmsii*, in line with the binary 0, 1 scoring system of the test.

The two way analysis of nitrate and pH (Fig. 7.4) shows an increased probability of *C. helmsii* presence at low nitrate levels. This is indicated by the darker vertical band on the left of *x*-axis. As nitrate levels increase at pH levels below 8, the probability of *C. helmsii* presence decreases. Above a pH level of 8, increases in nitrate show a relationship with an increased probability of *C. helmsii* being present.

Discussion

The comparison of water chemistry values between invaded and uninvaded sites showed no statistical difference using Mann-Whitney analysis. When using binary logistic regression, TON (Total Organic Nitrogen) was found to significantly differ between invaded sites, which had lower TON levels, compared to uninvaded sites, which had significantly higher TON levels. When comparing two factor combined variables, TON and pH were found to significantly differ between invaded and uninvaded sites. TON levels were lower on invaded sites up to a level of approximately pH 8, where it appeared to no longer show a relationship.

Single Variable Analysis

The measures of pH, conductivity, TDS, sodium and potassium were found not to be significantly different between the invaded and uninvaded sites. This may indicate that *C. helmsii* is able to establish and grow in a wide range of habitats with little preference. However, it is difficult to assess whether the sites sampled in this study are representative of a wide range of water chemistry values. Though different habitats and waterbody types were actively sought and included in an attempt to generate a wide range of values, whether this was achieved cannot be guaranteed. A national database of lentic waterbodies is lacking. The Countryside Survey Ponds Report (Williams *et al*., 2007) was the first study of the nutrient water chemistry of ponds in the UK, and remains the most current. As it is only a single recording of the surveyed waterbodies from a single sampling event, its use as a comparative is likely to be of low accuracy. If the average values for both TON and phosphorus for England are used as a comparative to the study of *C. helmsii* lentic systems, it can be seen that the *C. helmsii* systems provide data that is both above and below this level. This is also true for the in-field measured values of pH and conductivity. This spread of data either side of the values generated from the Ponds Report (Williams *et al*., 2007) does not however give clear evidence that the survey locations sampled in the *C. helmsii* study can be deemed as a wide range of habitats. Therefore, whether *C. helmsii* can be categorised as a true generalist, able to accommodate a wide range of habitats, remains likely but not definitive.

In an earlier study, low pH levels were thought to show a negative correlation towards the growth and establishment of *C. helmsii* (Brunet, 2002). Brunet's study did not find a clear distinction between the invaded sites and uninvaded control sites, which supports the findings from the current *C. helmsii* study. It was also thought that conductivity acted as a limiting factor towards growth, with values in excess of 300 µS cm^{-1} preventing growth of *C. helmsii* (Brunet, 2002). In this study, readings of up to 3601 µS cm^{-1} (Rye Street, location 2) were found, which supported *C. helmsii* growth. These results therefore disagree with the previous findings of the earlier study on conductivity. It can now be concluded that *C. helmsii* can grow in waterbodies with far higher conductivity levels then was previously known.

The measurements of ammonia and phosphate were found not to be significantly different between invaded and uninvaded sites. Brunet (2002) found phosphorus to be a limiting factor to growth of *C. helmsii* within New Forest ponds. In the current study, the data does not show a clear distinction between phosphorus and *C. helmsii* growth. Phosphorus values up to 6.41mg l^{-1} were recorded, which is in excess of those is found in Brunet's study. Multiple sites

were also found to have no detectable phosphorus present. It can therefore be concluded that these results do not support the findings of Brunet's study. However, whilst the current data was collected from a wide range of sites, Brunet's results were found just in woodland ponds within the New Forest, whereas the current *C. helmsii* study incorporated ditches adjoining agricultural fields. These two habitats are likely to differ in terms of nutrient inputs, and so a difference between the two is not unexpected.

Binary Logistic Regression – Single Variable

A relationship between decreased TON and presence of *C. helmsii* was found by the regression analysis. This would seem to disagree with the general theory of invasive species being associated with habitats that have an artificially increased nutrient burden (Burke and Grime, 1996; Lake and Leishman, 2004; James, *et al*., 2010). It does however support the findings of Chen *et al*., (2010), who found that *Eichornia crassipies (*Water Hyacinth - another invasive aquatic species) was an efficient accumulator of nutrients, and capable of remediation of waste water.

Invaded sites may have a decrease in TON, and show a significant difference compared to uninvaded sites, due to an increase in primary productivity through the rapid growth of *C. helmsii*. Wetland plants have been shown to remove nitrogen from a system in previous studies (Scott *et al*., 2008; Hefting *et al*., 2012; Nazaret *et al*., 2013). This occurs by processes such as volatisation, denitrification, plant uptake and sedimentation (Reddy and DeLaune, 2008). In a study of invasive species in the Great Lakes, USA, it was shown that net primary productivity and standing biomass increased when nutrient levels were increased (Callaway *et al*., 2004). Invasive species have been shown to have greater net primary productivity when compared to natives (Angelonia *et al*., 2006). Dassonville *et al*. (2008) found enhanced nutrient uptake and above ground biomass for seven non-native species across 36 different sites of a range of soil and vegetation combinations. For *C. helmsii*, it may mean that it is better adapted to utilise the available nutrients within the water, rapidly increasing in biomass at the detriment to native species. This would result in the removal of available nitrogen from the system and thereby lower its recordable level within the water.

The effects of invasive species on nutrient levels are likely to be species and site specific. A study of 56 invasive species found that increases in biomass and net primary productivity occurred simultaneously with increases in nitrogen availability (Ehrenfeld, 2003). This was not

always found to be true however, with the same species often having different effects dependant on the site studied, which was thought to be due to species composition and site water chemistry (Ehrenfeld, 2003). An increase in nitrogen availability caused by invasion may enable increases in establishment and growth (creating a positive feedback loop), but there is unlikely to be an in-exhaustive supply of nitrogen. Most of the sites studied had a well-established cover of *C. helmsii*, and therefore may have already begun to exhaust the supply of nitrogen available, thus giving the reduced levels shown by the analysis. Further studies of sites under different levels of invasion may provide evidence as to whether this reduction in available nitrogen is occurring over time.

C. helmsii was found to favour nutrient enriched rich waters when studied in tank trial experiments (Hussner, 2009). It was proposed that it would grow faster and produce more populations of denser growth in more eutrophic waters. In the current study, it would appear that the opposite is occurring. *C. helmsii* appears to be showing a preference for low nitrogen sites. What cannot be distinguished is what the nutrient status of the site was before invasion, with modifications of the nutrient levels after invasion not being representative of pre-invasion levels. Dassonville *et al*. (2008) found that the growth of non-natives correlated with the site conditions for a range of chemicals and nutrients before establishment of the non-native, and so felt increased biomass was caused by an increased availability of initial nutrients. Liao *et al*. (2008) however found that this clear distinction is not apparent, and that a large degree of variability exists. As no other chemical variable was significantly different between the invaded and uninvaded sites, and no obvious explanation can be seen as to why nitrogen would be higher on invaded sites, it would seem more likely that nitrate levels have been altered after colonisation by *C. helmsii*, rather than invasion occurring due to increased nitrate level.

Answering this question in the field directly would not be practical, as it would require sites to be intentionally stocked with the plant, with no guarantee of its removal after the trials due to the limited control techniques available. A development on Hussner's (2009) trial experiments would be to use experimental mesocosms within natural systems, which would receive water from these systems but would not be able to release reproductive propagules. The experimental results from Hussner using tank trials may only provide evidence for increased macrophyte growth with nutrient enrichment, which would likely occur for most aquatic macrophytes.

Nutrient increase has not always been found to be associated to the success of invasive species. A study of natural and agricultural *Vaccinium sp.* (Cranberry) bogs in Massachusetts,

USA, found no link between increased nutrient levels and non-native species (Sandler *et al*., 2007). Sandler *et al*. (2007) also studied edge effects, as they were thought to be more vulnerable to increased nutrient from agricultural activity. Whilst edges were found to have greater numbers of introduced species, nutrient input was thought not to be the cause, with propagule pressure and artificial disturbance being more likely. Increases in fertiliser application were shown not to increase the establishment of invasives in a study of grassland sites in Romania (Eszter, 2012). Nutrient additions on two grassland sites had no impact on the establishment of invasive seed species from seed inoculations, with seed size shown to be more important (Eszter, 2012). This illustrates that the effect of nutrients on the establishment of invasives is likely to be a combined effect with propagule type and morphology, as opposed to just nutrient availability

The size of *C. helmsii*, and its minimal dieback during the winter, may prevent nitrogen from cycling back into the water and soil through decomposition. A study of wetland invaders showed that a positive increase can occur in nitrogen levels when large invaders occupy a site, which produce large amounts of organic material for decomposition (Curriea, 2014). Large invaders increased the N-cycling process by 23% compared to native systems, increasing net primary productivity by 33%. Smaller invaders were found not to exist at high nitrogen inflow sites, and never dominated compared to other wetland native species. It was thought that this process may be due to the decreased augmentation of N-cycling effects during decomposition (Curriea, 2014). If this is considered in the example of *C. helmsii*, which also shows minimal decomposition rates, increases in N-cycling is likely not to be enhanced. A net decrease in nitrogen may therefore occur, with its use in plant tissues and growth removing it from the sites nutrient cycle.

This theory suggests nutrient input into the water bodies studied was not continuous, and that it was acting as a limiting factor to growth. The species better able to utilise the available nutrient would therefore have a competitive advantage when compared with competing species. *C. helmsii* has adaptations, such as CAM metabolism (Newman and Raven, 1995; Maberly and Madsen, 2002) that may explain why it is able to grow and increase in abundance more rapidly than its native competitors. Chapter 4 has shown that macrophyte diversity changes do occur, but not in a manner so as to lead to site degradation or species losses. *C. helmsii* may be being prevented from creating monocultures due to a limit on the available nitrogen within the waterbody after its initial rapid expansion and growth. Further analysis of the studied water bodies over time would be required to determine whether nutrient input to

a site is occurring continuously. If inputs are limited, it may be that reduced TON levels are a factor limiting the growth of *C. helmsii*, which is subsequently removing nitrogen from the system.

Binary Logistic Regression – Two Variables

The relationship that was found when comparing the two way effects of TON and pH may be due to the reduction in available H^+, used for increases in plant protein development and subsequent growth (Taiz and Zeiger, 2006). Over time in static waters these may begin to be used up. The balance between use for plant tissue growth and recharge from surface waters and/or horizontal percolation through the soil and substrata is likely to become unbalanced (Dussart, 1985). The removal of protons would therefore lead to an increase in pH in relation to decreases in organic nitrogen.

C. helmsii grows rapidly which uses nitrate for growth, and may explain why this relationship is stronger on invaded sites and not on control sites. The addition of lime, and therefore an increase in pH, has been shown to increase plant growth and nitrate utilisation on eutrophic wetlands (Gonzalez-Alcaraz *et al*., 2013). This was thought to be due to interactions with microbiota around the roots of macrophytes, with enhanced denitrification to the atmosphere (Gonzalez Alcaraz *et al*., 2013). This is an area that would require continued study of *C. helmsii*, in order to ascertain whether changes over time occur on carbonate rich sites like those found in Kent (Toghill, 2002). The cumulative effects of this possible change to a site pH may have impacts on other species such as macrophytes and macroinvertebrates.

A further mechanism that may explain the increase in pH is the ability for *C. helmsii* to carry out CAM photosynthesis. The uptake of dissolved inorganic carbon (DIC) is suggested as an advantage of CAM species in aquatic habitats in the form of bicarbonate (HCO_3^-) (Newman and Raven, 1995; Maberly and Madsen, 2002). CAM activity in *C. helmsii* has been shown to increase between April to July (Klavesen and Maberly, 2009). Prolific growth of *C. helmsii* may result in uptake of CO_2 during the day, with continued uptake of CO_2 through CAM as well as bicarbonate at night. The continual removal would lead to an increase in pH through the subsequent removal of H^+ ions for tissue growth, at the same time as removal of CO_2. It is not restricted just to CAM species however, as it has also been shown that the uptake of the carbonate ion HCO_3^- can also occur in aquatic species not exhibiting CAM metabolism (Taiz and Zeiger, 2006). This has shown to occur in freshwater ponds that have high primary production (Tucker and Abramo, 2008).

The significant combination of the two-way effect of pH and TON may also be linked to changes in soil microbiology. It is likely that soil microbiology is associated with set pH parameters and microbiota, including that of nitrogen fixing biota, which may be affected by invasion. Evidence exists illustrating the changes to soil micro biota by invasives, as an enhancement to nutrient availability. In some situations, native species have been shown to compete well with invasives under nutrient limited conditions, due to the presence of mycorrhizal symbionts. Holdredge *et al*. (2010) found that nutrient addition increased above ground growth of invasives by 67% (biomass), and below ground root growth was 27% greater for lateral expansion. This was felt to be due to the symbiotic effects of co-evolution of both native macrophyte species and their symbiotic associate mycorrhizae. Though this may appear to give native species an advantage, it has shown how invasives are able to modify the soil microbiology over time with continued invasive presence. Angelonia *et al*., (2006) found that *Typha x glauca* (Cattail) was able to create additional microbiota niches, thereby supporting a greater range of species. This was linked to an increase in ammonia, nitrates and phosphates on invaded sites compared to sites dominated by native species, and was thought to be responsible for the success of the invasive species. The invasive macrophyte species *Centaurea maculosa* (Spotted Knapweed) was shown to be responsible for increases in soil microbiology, thus providing beneficial conditions for its own growth and thus perpetuating a positive feedback loop (Callaway *et al*., 2004). This is supported by Rodgers *et al*. (2008), who also found a potential positive feedback situation with *Alliaria petiolata* (Garlic Mustard) increasing decomposition rates of leaf litter, thereby providing more nutrients to stimulate growth. The interaction between an increase in nutrients and changes to soil microbiota have been shown to favour non-native species (Scharfy *et al*., 2010). Perkins and Nowak (2013) found that non-native grass species modified the plant-soil feedback to benefit the non-native grass, whilst native grasses modified the plant-soil feedback to the benefit of other native species. This may explain how some non-natives are able to establish more quickly than native species, by altering the habitat in their favour to increase nutrient collection.

To date no investigations into the effects of invasion by *C. helmsii* on soil and water microbiology have been conducted. Studies of effects of *C. helmsii* on the soil microbiology would enable greater understanding of nutrient cycling within the invaded system, and may provide support for the data shown by this study. It may provide a greater understanding of the effects of invasion not just on the macro-scale communities, but on the microbiology of sites. This is likely to be impacted by invasion, with a co-evolution between both native macro and micro fauna and flora possibly explaining why TON levels have shown significant effects.

The nutrient levels across all of the sites were likely to be at their lowest, due to the time of year that the sites were surveyed. The surveying time of June-August correlates to the most active growing period of the macrophyte species present on the sites, and therefore the nutrient pool is likely to have been weighted towards storage within plant tissues. To better understand the flux of nutrients throughout the year, further surveying would be beneficial across a full season, to accommodate for decomposition and the movement of nutrients back into the waters and soils.

Conclusion

A comparison of single water chemistry variables of sites invaded by *C. helmsii* compared with control sites showed no statistically significant results. The growing conditions of pH, conductivity and TDS that were measured in the field showed no distinct patterns or relationships between the two types of site, and so the ability to predict sites of future invasion remains difficult. The values of conductivity expanded above the highest previous published limit form the only other published data available. Analysis of sodium and potassium found no statistical relationships between invaded and uninvaded sites. Binary logistic regression illustrated a relationship for both TON (total organic nitrogen) and the combined variables of TON and pH. Increased primary productivity leading to a decrease in available nitrate was thought a possible explanation. Changes to the microbiota of the soil and water, found to occur in studies of many other invasive species were also offered as possible explanations for these significant results.

Though the aim of this study was to provide a dataset of many types of waterbody from a range of habitats, an expansion in the dataset size would likely improve accuracy. A system of monitoring throughout the year may also help to understand how the nutrient fluxes change within the invaded systems, which may either support or contradict the theory of reduced nutrient levels due to increases in primary productivity. A microbiological assay of the water and soils of the sites may also aid in understanding how invasion by *C. helmsii* modifies the microbiology, if at all. This would likely require a long term sampling effort over many years, as the literature suggests modifications and symbiotic relationships between invasives and micro biota occur over time. Prolonged invasion is likely to provide the selection pressure necessary to promote this form of co-evolution between the two.

Chapter 8 – Study Conclusion

Overview of Findings

The study was divided into 5 key areas that attempted to answer the research question of whether any measurable impact of invasion by *C. helmsii* could be found. The first was an analysis of spread patterns using GIS models and existing datasets of *C. helmsii* distribution, in chapter 2. Chapters 4 and 5 investigated the effects of invasion on macrophyte and macroinvertebrate species. The effects on soil seed bank composition and the impact that management attempts has had on them was explored in chapter 6. Water chemistry was studied in chapter 7, which included both metals and nutrients from invaded and control sites. The specific findings of each of these subject areas were discussed within the chapters, with the key findings highlighted below.

Spread Patterns

The investigation into mapping the spread of *C. helmsii* used pre-existing data taken from databases of records and those published in the literature. The results showed that the initial spread was through long distance dispersal until the 1980's, and possibly due to distribution through the horticultural trade. This is supported by the literature, which describes how the plant was initially introduced into the country through an ornamental plant supplier, and was sold as an oxygenating plant (Laundon, 1961). After the 1980's, the dispersal pattern changed to be predominantly short-distance dispersal, after a likely establishment phase and sufficient establishment into native species assemblages. After this point, dispersal could have been due to natural vectors (Carlquist, 1967; Figuerola and Green, 2002,), as well as through disturbance through control programmes. This may suggest that the control of the plant could have been encouraging spread, though the increased surveying effort that might be associated with the discovery of novel species could also explain this. Though the model suggests that SDD became more influential, the plant was still readily available even up until the start of this study, and so could still have been dispersed through the horticultural trade. This is supported by the findings of the more localised study of the Kent data, which found a relationship between the *C. helmsii* records and the distance to settlements. As dispersal from gardens could have been

one of the causes of spread into naturalised settings, this relationship would appear to be supported.

Macrophytes

The investigation of the impacts of invasion on other macrophyte species found no significant difference in the number of macrophyte species present between invaded and uninvaded sites. It would therefore seem that *C. helmsii* does not decrease native macrophyte species numbers.

When comparing average rarity scores, which were found by scoring the macrophyte species present using databases of rarity both at a national and at a county level, some results were found to be significant. For 4 of the 5 scoring systems (2 national and 2 county level), average rarity scores were found to be higher on invaded sites than on uninvaded control sites. This would appear to indicate an effect of invasion, though not what may have been initially expected. Invasion would appear to be facilitating the establishment and growth of rarer species, which could be due to the removal of dominant species that would have prevented the establishment of these species without the presence of the invasive. It is not possible to tell from this data whether it is *C. helmsii* that has caused this change, or if there was any impact on the habitat that facilitated invasion by *C. helmsii* initially. However, when considering the question of whether invasion does have an impact, these results suggest that it is linked to a change in the composition of species, which could be either beneficial or detrimental to the ecology of the habitat.

When these results were subdivided into habitat type and waterbody type, changes to macrophyte composition were also found under certain combinations, with *C. helmsii* invaded sites showing higher scores for rarity than control sites. These changes were most obvious when county level woodlands were isolated from the data, and so would be a prime candidate for further investigation. Management activity was not found to be influential to macrophyte presence, and so when using this set of data, control activities cannot be seen to be having an adverse effect on native species.

Freshwater Macroinvertebrates

The investigation of freshwater macroinvertebrates found that overall, no evidence of significant differences were found between invaded and uninvaded sites. Species number and species scores (derived from both pollution derived and diversity derived indices) were found not to differ with invasion. Subdividing the data into habitat, waterbody type and management effort also found no significant difference. Therefore no measurable impact of invasion by *C. helmsii* on macroinvertebrates can be found from the data.

Seed Banks

Invasion by *C. helmsii* was not found to have any effect on the seed banks when compared between invaded and uninvaded sites. There was also no link between the above ground macrophyte species present, and the below ground seed banks. The attempt to find a method of analysing seed banks in this manner (by using macrophytes as a representation of the seed bank) was therefore found not to be accurate and not applicable. When comparing the length of invasion by *C. helmsii* with the seed bank data, no correlation could be found. A compounded effect of prolonged invasion by *C. helmsii* on the seed banks, and subsequent impact on native decolonisation by natives, could not be found. The division of the data into management effort found that seed bank diversity was significantly reduced on sites that were managed, compared to those that were either target managed or left unmanaged. The act of managing *C. helmsii* is therefore having an impact on the seed banks and may be affecting the ability for native species to recolonise.

Water Chemistry

Comparisons between invaded and uninvaded sites were found not to have significantly different water chemistry values using non-parametric tests. The use of binary logistic regression found that increased total organic nitrogen levels related significantly with uninvaded sites, as did the 2-way interaction of pH and total organic nitrogen. This would appear to show that invasion may be having an effect on the water chemistry of the invaded sites, or it may be showing a preference towards lower nitrogen habitats. Chapter 7 discusses these factors in more details, but concludes that the most likely answer is that the rapid

growth of *C. helmsii* is removing nitrogen from the waterbody. This effect on the habitat could have implications for the ecology of the waterbodies, but would require further exploration.

Implications of the study

No conclusive published data currently exists that provides evidence that *C. helmsii* causes a loss of species diversity and a decline in ecosystem functioning. However, the emphasis of both the literature and action in the field has been of large-scale control programs that attempt to remove the plant. In doing so, it is likely that both the habitats and native species have been impacted by these actions, as control methods are generally not target-specific (Dawson and Henville, 1991; Spencer-Jones, 1994; Child and Spencer-Jones, 1995; Leach and Dawson, 1999; Bridge, 2005; Gomes, 2005).

C. helmsii is listed on schedule 9 of the Wildlife and Countryside Act 1981, which legislates against intentional release into the wild or intentional propagation in the wild. Therefore, no legal responsibility exists to control the plant. Land managers are likely to be carrying out control programs due to guidance set out by government bodies, which are not backed up with evidence (RPS Ecoscope Applied Ecologists, 2005; Non-Native Species Secreteriat, 2015). There may also be pressure applied to land managers and organisations to control the plant from members of the public, who assume that an impact is being caused by *C. helmsii*, due to its obvious change to the visual representation of the waterbody (James, 1995; Verbrugge *et al*., 2013). These anecdotal and observational representations of *C. helmsii* growth do have a level of truth, as the cover and abundance of species are likely to be altered by rapid growth of *C. helmsii*. However, this study has shown no evidence of an impact upon macrophyte species numbers, with the compositional changes resulting in rarer species being present being unlikely to be noticed unless surveyed in detail.

Macroinvertebrate species number and diversity and the seed banks of invaded sites have also been shown to not be affected by *C. helmsii* growth in this study. The act of control has been shown to be detrimental towards seed banks, and the positive effect of *C. helmsii* in removing nitrogen from the water which has the potential to cause changes through excess nutrient presence has also been illustrated. Therefore, the need to control the plant, which requires high levels of resources and which has little effect when considering successful removal of *C. helmsii,* should be reconsidered on a site selective basis. This supports the findings of Langdon (2004), who found no measurable impact of *C. helmsii.*

Control of non-native plants without evidence of impact towards native species has been shown to occur for other non-native species. The control of *Lythrum salicaria* (Purple Loosestrife) was carried out on a wide scale and at great expense in the USA, with little scientific research showing evidence for the need to control it (Lavoie, 2010). Gardener *et al*. (2010) showed how the resources directed at the control of *Martynia annua* (Devil's Claw) over a 20 year control strategy were disproportionate both to the effects of the species on natives, and the overall changes to its distribution. Non target effects have also been shown where control of one non-native species facilitates the establishment of another (Skurski *et al*., 2013). Effects of invasions on macroinvertebrates have also shown not to occur in studies of *Phragmites australis* (Common Reed) (Siersma *et al*., 2007) and *Fallopia japonica* (Japanese Knotweed) (Bottolier-curtet *et al*., 2007).

The results of this study should not be seen as a definitive example of the action of invasives species, as it has been shown that different species can have varying effects on the same study site (Pysek and Pysek, 1995; Hejda and Pysek, 2006). It has also been shown that when invasives are studied and measured for effects on native species, control may be required in an attempt to prevent species losses (Hulme and Bremner, 2005). What it does show, however, is that an evidence-based approach to control is key to directing limited resources effectively, otherwise a lot of time and money could be wasted in controlling a species that is having no measurable impact.

Current research is centred upon control, with the development of a biological control agent (CABI, 2014). Whilst this would make the control target specific, therefore reducing any indirect effects towards native species, biological control has been shown to not always be a successful option due to the complexities of ecosystem change that they cause (Messing and Wright, 2006). Whilst this method of control may be both more attractive due to reduced impact, and more successful than current methods, it is likely to take some time to develop to the point that it can be used in the field. By this time, the large scale growth and integration of *C. helmsii* into native habitats may have changed, as has occurred for other non-native species after a sudden initial rise in population size which was followed by a rapid collapse in population size (Hawkes, 2007; Simberloff and Gibbons, 2007; Dostal *et al*., 2013). This may be especially relevant for *C. helmsii*, which has been shown to have minimal genetic variability from previous studies (Dawson, 1994).

Limitations of the study and future work

The study used measures of diversity rather than measuring percentage cover, which is a method that has been carried out in previous studies on other species (Linton and Goulder, 2000; Williams *et al*., 2003; Nicolet *et al*., 2004; Smith *et al*., 2006; Cereghino *et al*., 2008; Gerber *et al*., 2008). Percentage cover was felt to be an inaccurate method of measuring species distributions, as *C. helmsii* is able to grow in dense stands giving a 100% cover score, with other species still able to grow amongst it. Due to its numerous growth forms and differences between above and below water growth (Dawson and Warman, 1987), cover differs dependant on which level of the water strata is studied. An adaptation to this would be to consider the level of growth of *C. helmsii* in distinct bands. These could be 'well established', 'present but not dominant' and 'absent-control'. This type of categorisation has been used successfully on studies of other non-native species (Fisher *et al*., 2009). This may allow the sites and therefore data to be segregated in a more representative way which take account of the 'dominance' of *C. helmsii* within the habitat.

By arranging the data into independent sampling locations and pooling the results, the analysis assumes that each site was independent. Whilst every effort was made to ensure that this was the situation in-field, with each waterbody being separated, a level of error may have been included due to this pooling of the data. An alternative to this would be to analyse each site independently, rather than pooling the data. This would be a viable option for some locations, but not for all of them. Due to the level of spread exhibited by *C. helmsii*, some sampling locations had no control areas (areas free from *C. helmsii* growth) available to survey. This would need to be factored in to further analysis, and would rely on locating survey sites that contained both invaded waterbodies and those free from the species. Whilst additional sites were added in that were similar in location and environmental conditions, the assumption that they acted as true controls may be flawed.

Some of the P-value significance levels had values close to $P<0.05$, such as the seed bank comparison of management which had a significance value of 0.0391. Due to the number of analyses carried out, and the number of different combinations that were applied to the analyses, this level of significance may need to be reconsidered. By increasing the number of analyses, the probability of finding a significant result increases. This therefore makes the significance level at the 5% level (0.05) less robust than for single comparisons. A way of factoring this into the analysis would be by adjusting the significance level using the Bonferonni correction. This acts to reduce the significance level in relation to the number of

comparison carried out (for example, 10 comparisons would result in an adjustment of 0.05/10, and therefore a new significance level of 0.005 (0.5%). The use of the Bonferonni correction would allow for greater confidence to be placed on the results of the analyses.

The freshwater macroinvertebrates were identified to family level only. More detail, and therefore the exploration of an impact by *C. helmsii* to a greater depth, would be found by identifying the specimens to species level. This would also enable Red Data Book species to be identified, which would likely change the rarity scorings for the sites and therefore may give a greater weighting towards rarities and their presence. This would also enable the use of the Chadd and Extence (2004) diversity method, which requires both identification to species level and the use of rarities as a multiplier, to be applied to the data. More surveys, across the entire surveying season (from March to October) would increase the range of species found. Different life cycles may have meant that certain species were not present during the summer surveying that was carried out in this study.

A single soil core was taken to analyse the seed banks of each of the survey locations. A more accurate representation of the seed bank would be provided by collecting multiple samples from the same site, and pooling the soil cores. This would likely require a reduction in the large number of sites visited, with a change of emphasis from a broad ranging surveying strategy across multiple habitats and waterbodies, to a narrower range of sites that could be studied in more detail. This is applicable to all of the surveying methods carried out in the study.

Examination of seed viability could be further enhanced by using the seedling emergence method (Roberts, 1981, Ter Heerdt *et al*., 1996). Chapter 6 used visual methods for identifying seed viability, including a pressure test of the endosperm. Whilst this is likely to have removed any seeds that were unlikely to germinate, seedling germination is likely to be a more accurate way of assessing this. Roberts (1981) discusses the procedures for this, including its short fallings when compared to visual identification. These include the provision of only one set of light and water conditions as a generic 'germination' attempt, which may exclude certain seed species and promote others. A limit on resources for germinating large numbers of seeds would also have been experienced. The requirement for large amounts of greenhouse space may be reduced by using the concentrating methods developed by Ter Heerdt *et al.* (1996) using a concentrating method, which removes excess soil by washing.

Chapter 7 identified that TON (total organic nitrogen) levels were significantly lower on sites where *C. helmsii* was present. What cannot be interpreted from the data is whether this

reduction was before the plant established, or whether it was caused by increased growth of the plant tissues. Hussner (2009) found that *C. helmsii* grew rapidly when nutrients were increased in tank trials, but this is unlikely to show what is happening in the field with the range of environmental stochasticity that *C. helmsii* is exposed to. This should be further explored to determine whether competition between natives and *C. helmsii* results in nutrient changes that favour the growth of the non-native, or whether the field situations are caused due to nutrients being in excess and therefore no competition for resources. The use of in field mesocosms could be used, but bio-security would be an integral factor to incorporate into the study.

Another additional chemical variable that could be measured is the oxygen levels of the waterbodies. This was not carried out in the field due to the lack of available equipment, but would be an easily measured additional result. As growth of *C. helmsii* often reduces the amount of open water, thereby decreasing the water/air boundary layer, reduced oxygen levels may occur. This may however be balanced by *C. helmsii* being sold initially as an oxygenating plant in the aquatics trade (Laundon, 1961). Measures of open water, area of cover and oxygen levels would be required to compare whether this is occurring.

To identify the long term changes to a habitat, sites invaded by *C. helmsii* earlier than those in this study could be measured for effects. This would require expanding the survey outside of Kent and East Sussex, but would allow for sites to be studied that showed a longer residence time of the non-native plant. Issues with this would involve knowing the history of the management of the site, as it is unlikely that it has been left untouched for the length of time that *C. helmsii* has been present. This may be difficult, as land managers will almost certainly have changed over the time of it being present (as was experienced in this study), and so would rely on detailed documentation. Land ownership may also have changed, with the waterbody no longer being present due to a change in land use, and therefore presence of *C. helmsii* would most likely have been lost also. Its position as a rarity however may have promoted record keeping, and so could be explored to see whether it has integrated into the habitat with less dominance than was found in this research. This has been shown to occur for other species (Hawkes, 2007; Simberloff and Gibbons, 2007; Dostal *et al*., 2013).

Further genetic investigations of *C. helmsii* should be carried out to ascertain definitively whether the source of the plant is from a single location, as is described in previous work (Laundon, 1961; Dawson and Warman, 1987). Dawson (1994) found in his study that all of the evidence indicated this to be true, but consisted of a small sample set. By increasing the

number of plants tested, across a larger geographic range, this can be further explored to either agree with or contradict the previous results. This is especially important as the ability for *C. helmsii* to grow from seed has been recently questioned (Denys *et al*., 2014), which could lead to sexual reproduction and the ability to adapt.

Conclusion

No measurable negative effect could be found towards native species on sites colonised by *C. helmsii*. The act of management has shown to be detrimental to native species when seed banks are quantified and compared between managed and unmanaged sites. A possible benefit of *C. helmsii* growth - the uptake of total organic nitrogen has also been found. This could have uses in pollution control that could otherwise have detrimental effects upon the habitat.

The need to control *C. helmsii* on all sites where it is found should be questioned. The use of initial surveys to assess whether action is needed should be carried out before control attempts are made. This study only took account of sites in the south east of England, and so should not be transferred to other locations without first carrying out comparative surveys between invaded and uninvaded sites.

The generally accepted viewpoint of *C. helmsii* is that it requires removal to prevent the loss of native species. This study did not find this to be the case. The resources that are invested in the control of this species would likely be better spent elsewhere, to generate greater conservation and ecological gains. Further research, both on this species and other non-native species where data is lacking, should be conducted to discover the true impact of invasive species beyond what is often anecdotal and inaccurate.

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Appendix 1 - Site Classifications

Habitat Type

Coastal		**Lake**	
Oare 1	Oare C1	Bough Beech 1	Bouch Beech C1
Oare 2	Oare C2	Bough Beech 2	Sevenoaks C1
Oare 3	Dungeness C1	Bough Beech 3	Fowlmead C1
Oare 4	Rye Street C1	Bough Beech 4	Fowlmead C2
Oare 5	Rye Street C2	Bough Beech 5	Fowlmead C3
Dungeness 1	Romney Marsh C1	Bewl 1	
Dungeness 2	Romney Marsh C2	Bewl 2	
Dungeness 3		Bewl 3	
Dungeness 4		Bewl 4	
Dungeness 5		Bewl 5	
Rye Street 1		Sevenoaks 1	
Rye Street 2		Sevenoaks 2	
Rye Street 3		Sevenoaks 3	
Rye Street 4		Sevenpaks 4	
Rye Street 5		Sevenoaks 5	
Rye Harbour 1			
Rye Harbour 2			
Rye Harbour 3			
Rye Harbour 4			
Rye Harbour 5			
Woodland			
Perry 1	GW Oare 1		
Perry 2	GW Oare 2		
Beacon Wood 1	GW Oare 3		
Beacon Wood 2	Orelestone C1		
Blean 1	Orlestone C2		
Blean 2			
Blean 3			
Eastry 1			
Eastry 2			
Shorne 1			
Shorne 2			
Shorne 3			
Shorne 4			
Shorne 5			

Management Effort

Active Management	Unmanaged/Target management
Hothfield 3	Oare 1
Hothfield 4	Oare 2
Shorne 1	Oare 3
Shorne 2	Oare 4
Shorne 3	Oare 5
Shorne 4	Bough Beech 1
Shorne 5	Bough Beech 2
Dungeness 1	Bough Beech 3
Dungeness 2	Bough Beech 4
Dungeness 3	Bough Beech 5
Dungeness 4	Bough Beech F1
Dungeness 5	Bough Beech F2
Sevenoaks 1	Bough Beech F3
Sevenoaks 2	Perry 1
Sevenoaks 3	Perry 2
Sevenpaks 4	Beacon Wood 1
Sevenoaks 5	Beacon Wood 2
Rye Street 1	Blean 1
Rye Street 2	Blean 2
Rye Street 3	Blean 3
Rye Street 4	Bewl 1
Rye Street 5	Bewl 2
Rye Harbour 1	Bewl 3
Rye Harbour 2	Bewl 4
Rye Harbour 3	Bewl 5
Rye Harbour 4	Eastry 1
Rye Harbour 5	Eastry 2
Stodmarsh 1	
Stodmarsh 2	
Stodmarsh 3	
Stodmarsh 4	
Stodmarsh 5	

Waterbody Type

Ditches	Lakes	Ponds 1	Ponds 2
Oare 1	Bewl 1	Hothfield 3	Perry 1
Oare 2	Bewl 2	Hothfield 4	Perry 2
Oare 3	Bewl 3	Shorne 1	Beacon Wood 1
Oare 4	Bewl 4	Shorne 2	Beacon Wood 2

Oare 5	Bewl 5	Shorne 3	Blean 1
Bough Beech 5	Sevenoaks 1	Shorne 4	Blean 2
Dungeness 1	Sevenoaks 2	Shorne 5	Blean 3
Dungeness 2	Sevenoaks 3	Bough Beech 1	Eastry 1
Rye Street 1	Dungeness 3	Bough Beech 2	Eastry 2
Rye Street 2	Dungeness 4	Bough Beech 3	Stodmarsh 1
Rye Street 3	Dungeness 5	Bough Beech 4	Stodmarsh 2
Rye Street 4		Bough Beech F1	Stodmarsh 5
Rye Street 5		Bough Beech F2	Rye Harbour 3
Rye Harbour 1		Bough Beech F3	Rye Harbour 4
Rye Harbour 2		Sevenpaks 4	
Rye Harbour 5		Sevenoaks 5	
Stodmarsh 3			
Stodmarsh 4			

Appendix 2 – Sampling Locations

C. helmsii Locations

Invaded Locations	LAT	LONG	ELEVATION	GRID REF	X	Y
Oare 1	51.344225	0.878578	-0.46328	TR0057464505	600574	164505
Oare 2	51.344222	0.87869	0.212772	TR0058264505	600582	164505
Oare 3	51.3443	0.878947	6.035686	TR0060064514	600600	164514
Oare 4	51.344265	0.878881	1.962616	TR0059564510	600595	164510
Oare 5	51.344282	0.879119	2.576855	TR0061264513	600612	164513
Hothfield 3	51.181485	0.815596	68.57753	TQ9688346240	596883	146240
Hothfield 4	51.181193	0.815612	67.502678	TQ9688546208	596885	146208
Shorne 1	51.40397	0.418887	110.133499	TQ6834569992	568345	169992
Shorne 2	51.403961	0.418681	107.383743	TQ6833069990	568330	169990
Shorne 3	51.403109	0.41791	111.72924	TQ6828069894	568280	169894
Shorne 4	51.405545	0.417727	104.555511	TQ6825870164	568258	170164
Shorne 5	51.405807	0.421576	107.280907	TQ6852570202	568525	170202
Bough Beech 1	51.224378	0.138608	65.890663	TQ4943449415	549434	149415
Bough Beech 2	51.22476	0.138372	62.898945	TQ4941649457	549416	149457
Bough Beech 3	51.224379	0.139339	69.216827	TQ4948549417	549485	149417
Bough Beech 4	51.224147	0.139304	70.118835	TQ4948349391	549483	149391
Bough Beech 5	51.224402	0.139154	75.322983	TQ4947249419	549472	149419
Bewl 1	51.062757	0.421926	77.542679	TQ6980732059	569807	132059
Bewl 2	51.06268	0.421789	70.16758	TQ6979832050	569798	132050
Bewl 3	51.062326	0.41999	68.31134	TQ6967332007	569673	132007
Bewl 4	51.062391	0.418299	71.67601	TQ6955432010	569554	132010
Bewl 5	51.060706	0.422511	68.977814	TQ6985631832	569856	131832
Dungeness 1	50.935845	0.934432	-0.486034	TR0627619256	606276	119256
Dungeness 2	50.932928	0.931642	-2.995396	TR0609318924	606093	118924
Dungeness 3	50.926353	0.923525	-6.624657	TR0555118170	605551	118170
Dungeness 4	50.929273	0.920619	-4.004208	TR0533418487	605334	118487
Dungeness 5	50.927595	0.93666	-0.43906	TR0646918345	606469	118345
Sevenoaks 1	51.291246	0.184523	61.392166	TQ5241856945	552418	156945
Sevenoaks 2	51.290109	0.179503	69.329498	TQ5207256808	552072	156808
Sevenoaks 3	51.294123	0.182593	70.152367	TQ5227457261	552274	157261
Sevenpaks 4	51.292603	0.17753	68.366295	TQ5192657082	551926	157082
Sevenoaks 5	51.292589	0.177027	73.125961	TQ5189157079	551891	157079
Perry 1	51.264945	0.930235	96.415215	TR0452355833	604523	155833
Perry 2	51.264929	0.930184	110.465942	TR0452055831	604520	155831
Rye Street 1	51.468081	0.509743	10.783836	TQ7441977333	574419	177333
Rye Street 2	51.468215	0.509533	6.023605	TQ7440477347	574404	177347
Rye Street 3	51.467323	0.508606	-3.184189	TQ7434377246	574343	177246
Rye Street 4	51.465216	0.508157	2.645294	TQ7432077010	574320	177010

Rye Street 5	51.465585	0.506263	-1.283382	TQ7418777047	574187	177047
Rye Harbour 1	50.935357	0.742247	5.862026	TQ9277718682	592777	118682
Rye Harbour 2	50.935475	0.743048	4.984009	TQ9283218697	592832	118697
Rye Harbour 3	50.940039	0.740476	3.184479	TQ9263319198	592633	119198
Rye Harbour 4	50.939956	0.740203	6.150387	TQ9261419188	592614	119188
Rye Harbour 5	50.943849	0.738283	3.500862	TQ9246319616	592463	119616
Stodmarsh 1	51.315872	1.204963	5.080276	TR2343862296	623438	162296
Stodmarsh 2	51.315963	1.204452	-1.725487	TR2340262304	623402	162304
Stodmarsh 3	51.316165	1.204467	-2.267517	TR2340262327	623402	162327
Stodmarsh 4	51.315818	1.208347	-0.119404	TR2367462300	623674	162300
Stodmarsh 5	51.315538	1.206501	0.994118	TR2354762263	623547	162263
Beacon Wood 1	51.421486	0.284511	74.109253	TQ5893871639	558938	171639
Beacon Wood 2	51.421449	0.285329	63.515892	TQ5899571637	558995	171637
Romney Marsh 1	50.996023	0.958691	8.796272	TR0771126014	607711	126014
Romney Marsh 2	50.99621	0.958904	5.038425	TR0772526036	607725	126036
Blean 1	51.301641	1.029438	80.979553	TR1127460194	611274	160194
Blean 2	51.301731	1.02986	73.342621	TR1130360205	611303	160205
Blean 3	51.298215	1.013406	88.710144	TR1017359767	610173	159767
Eastry 1	51.239771	1.30721	8.99609	TR3094354152	630943	154152
Eastry 2	51.239616	1.307099	4.463131	TR3093654134	630936	154134

Control Locations

Control Locations	LAT	LONG	ELEVATION	GRID REF	X	Y
Oare C1	51.34421	0.878258	1.539062	TR0055264502	600552	164502
Oare C2	51.344073	0.878295	2.0159	TR0055564487	600555	164487
GW Oare 1	51.326869	0.876663	15.309628	TR0051762570	600517	162570
GW Oare 2	51.326565	0.875134	10.860718	TR0041262532	600412	162532
GW Oare 3	51.325835	0.873366	19.731098	TR0029262446	600292	162446
Orelestone C1	51.077799	0.836995	55.22747	TQ9882334770	598823	134770
Orlestone C2	51.077973	0.837657	49.650837	TQ9886934791	598869	134791
Hothfield 1 C	51.181098	0.814991	66.543671	TQ9684246196	596842	146196
Hothfield 2 C	51.180504	0.812399	63.011749	TQ9666446123	596664	146123
Hothfield C1	51.175608	0.826106	67.391068	TQ9764245615	597642	145615
Hothfield C2	51.175654	0.827026	69.013069	TQ9770745623	597707	145623
Bouch Beech C1	51.224198	0.139783	67.57795	TQ4951649398	549516	149398
Dungeness C1	50.936963	0.932876	0.340637	TR0616119376	606161	119376
Sevenoaks C1	51.293887	0.180918	67.648529	TQ5215857231	552158	157231

Rye Street C1	51.464703	0.514799	10.037151	TQ7478376969	574783	176969
Rye Street C2	51.466059	0.511277	11.065529	TQ7453377112	574533	177112
Fowlmead C1	51.240264	1.378249	3.536747	TR3589854432	635898	154432
Fowlmead C2	51.238583	1.381721	10.54694	TR3614954256	636149	154256
Fowlmead C3	51.238516	1.381734	1.354187	TR3615054249	636150	154249
Stodmarsh C1	51.315579	1.207278	1.019554	TR2360162270	623601	162270
Stodmarsh C2	51.314601	1.207175	2.429764	TR2359962161	623599	162161
Romney Marsh C1	50.996847	0.959122	8.587234	TR0773826107	607738	126107
Romney Marsh C2	50.996774	0.958878	10.821621	TR0772126098	607721	126098

Appendix 3 – Species Spread and Designated Sites Data

Species Spread

NBN Data Spread Patterns							
% Spread	5km	10km	15km	20km			
1960	0.00	6.25	6.25	25.00			
1970	2.22	8.89	22.22	22.22			
1980	1.71	11.95	21.95	30.24			
1990	13.47	58.78	77.14	83.67			
2000	11.81	52.78	65.97	70.83			
2010	7.69	51.28	66.67	74.36			
NBN Data Designated Sites							
	1950	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
LNR	0.00	0.00	0.00	0.73	0.41	0.69	0.00
LNR + 2	33.33	23.08	11.11	13.41	15.92	15.28	17.95
NNR	0.00	0.00	0.00	0.49	0.82	0.00	0.00
NNR + 2	0.00	7.69	4.44	5.12	4.90	2.78	0.00
SSSI	0.00	0.00	4.44	5.37	6.12	4.86	10.26
SSSI + 2	33.33	53.85	48.89	45.61	44.08	47.22	38.46
AONB	0.00	7.69	11.11	10.73	7.35	10.42	20.51
AONB + 2	0.00	15.38	20.00	19.76	9.80	18.06	23.08
CP	0.00	0.00	0.00	0.24	0.41	0.69	0.00
CP + 2	0.00	23.08	8.89	6.59	11.43	10.42	7.69
SAC	0.00	0.00	8.89	4.15	3.27	3.47	7.69
SAC + 2	0.00	7.69	0.00	0.73	21.63	18.06	7.69
BSBI Data Spread Patterns							
	5km	10km	15km	20km			
1960	5.56	5.56	8.33	11.11111			
1970	0.99	7.92	14.85	21.78218			
1980	12.30	23.14	35.90	47.6584			
1990	47.95	73.72	89.64	93.66438			
2000	58.20	80.94	90.83	94.20989			
2010	73.43	88.47	95.74	97.99499			
BSBI Data Designated Sites							
	1950	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
LNR	0.00	0.00	1.98	2.11	7.36	3.56	5.01
LNR + 2	0.00	19.44	17.82	22.31	29.37	22.98	26.57
NNR	0.00	2.78	1.98	0.64	0.60	2.77	2.01
NNR + 2	0.00	11.11	8.91	6.80	7.45	8.44	6.52
SSSI	0.00	11.11	14.85	12.21	15.15	22.32	19.30
SSSI + 2	20.00	44.44	62.38	55.19	62.33	64.90	63.91

AONB	0.00	8.33	8.91	10.28	8.82	9.71	14.29
AONB + 2	0.00	8.33	16.83	18.27	16.01	15.86	20.80
CP	0.00	0.00	0.99	0.92	2.23	2.83	4.01
CP + 2	0.00	5.56	5.94	10.01	11.39	12.61	15.79
SAC	0.00	0.00	13.86	7.07	8.22	14.84	10.03
SAC + 2	0.00	19.44	14.85	15.43	15.33	17.13	15.04

Designated Sites

NBN Designated Site Size Calculations								
		1950	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
Decadal	**SIZE (HA)**	3	16	45	410	245	144	39
LNR	38555	0	0	0	3	1	1	0
LNR + 2	1808155	1	3	5	52	38	21	7
NNR	93290	0	0	0	2	2	0	0
NNR + 2	71986	0	1	2	19	10	4	0
SSSI	1078984	0	0	2	22	15	7	4
SSSI + 2	6310262	1	7	20	165	93	61	11
AONB	1920903	0	1	5	44	18	15	8
AONB + 2	1192715	0	1	4	37	6	11	1
CP	43779	0	0	0	1	1	1	0
CP + 2	852777	0	3	4	26	27	14	3
SAC	1013657	0	0	4	17	8	5	3
SAC + 2	866018	0	1	-4	-14	45	21	0
BSBI Designated Site Size Calculations								
		1950	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
Decadal	**SIZE (HA)**	10	36	101	1089	1168	1658	399
LNR	38555	0	0	2	23	86	59	20
LNR + 2	1846709	0	7	16	220	257	322	86
NNR	93290	0	1	2	7	7	46	8
NNR + 2	813159	0	3	7	67	80	94	18
SSSI	1078984	0	4	15	133	177	370	77
SSSI + 2	7389246	2	12	48	468	551	706	178
AONB	1920903	0	3	9	112	103	161	57
AONB + 2	3113618	0	0	8	87	84	102	26
CP	43779	0	0	1	10	26	47	16
CP + 2	896555	0	2	5	99	107	162	47
SAC	1013657	0	0	14	77	96	246	40
SAC + 2	1879675	0	7	1	91	83	38	20

Appendix 4 – Plant Naming Authorities for Species Recorded

Scientific Name	Common Name	Naming Authority
Acer pseudoplatanus	Sycamore	L.
Aesculus hippocastanum	Horse chestnut	L.
Ajuga reptans	Bugle	L.
Alisma plantago-aquatica	Water plantain	L.
Alnus glutinosa	Alder	(L.)Gaertn
Anthemis arvensis	Chamomile	L.
Apium nodiflorum	Fools watercress	(L.)Lag.
Argentina anserina	Silverweed	Rydb.
Berula erecta	Water parsnip	(Huds.)Coville
Betula pendula	Silver birch	Roth
Betula pubescens	Downy birch	Ehrh.
Blechnum spicant	Hard fern	(L.)Roth
Bolboschoenus maritimus	Sea club rush	(L.)Palla
Callitriche stagnalis	Water starwort	Scop.
Caltha palustris	Marsh marigold	L.
Cardamine pratensis	Cuckoo flower	L.
Carex demissa	Yellow sedge	(Andersson)B.Schmid
Carex echinata	Star sedge	Murray
Carex nigra	Common sedge	(L.)Reichard
Carex paniculata	Tussock sedge	L.
Carex pendula	Pendulous sedge	Huds.
Carex remota	Remote sedge	L.
Carex riparia	Pond sedge	Curtis
Centaurium pulchellum	Lesser centaury	(Sw.)Druce
Ceratophyllum demersum	Hornwort	L.
Circaea lutetiana	Enchanters nightshade	L.
Cirsium arvense	Creeping thistle	(L.)Scop
Cirsium palustre	Marsh thistle	(L.)Scop
Convolvulus arvensis	Bindweed	L.
Crassula helmsii	Crassula	(Kirk)Cockayne
Crataegus monogyna	Hawthorn	Jacq.
Dipsacus fullonum	Teasel	L.
Eleocharis palustris	Common spike rush	(L.) Roem. & Schult.
Elodea canadensis	Pondweed	Michx.
Epilobium hirsutum	Great willowherb	L.
Fagus sylvatica	Beech	L.
Fraxinus excelsior	Ash	L.
Galium palustre	Marsh bedstraw	L.
Galium aparine	Cleavers	L.
Geranium robertianum	Herb robert	L.
Glyceria declinata	Reed grass	Br.
Glyceria fluitans	Float grass	(L.) R. Br.
Hedera helix	Ivy	L.
Hippuris vulgaris	Marestail	L.
Holcus lanatus	Yorkshire fog	L.
Hydrocharis morsus-ranae	Frogbit	L.
Hydrocotyle vulgaris	Marsh pennywort	L.
Hypericum elodes	Marsh St. Johns Wort	L.
Iris pseudacorus	Flag iris	L.
Juncus articulatus	Jointed rush	L.
Juncus effusus	Soft rush	L.
Juncus inflexus	Hard rush	L.

Lemna minor	Lesser duckweed	L.
Lemna trisulca	Ivy leaved duckweed	L.
Lotus corniculatus	Birds foot trefoil	L.
Lychnis flos-cuculi	Ragged robin	L.
Lycopus europaeus	Gypsywort	L.
Lysimachia nummularia	Creeping jenny	L.
Lythrum salicaria	Purple loostrife	L.
Mentha aquatica	Water mint	L.
Menyanthes trifoliata	Bogbean	L.
Myosotis scorpioides	Water forget me not	L.
Myriophyllum spicatum	Eurasian Water milfoil	L.
Nuphar lutea	Water lily	(L.)Sm.
Oenanthe fistulosa	Water dropwort	L.
Persicaria hydropiper	Water pepper	(L.) Delarbre
Persicaria amphibia	Amphibious bistort	(L.)Gray
Phalaris arundinacea	Reed canary grass	L.
Phragmites australis	Common reed	(Cav.) Trin. ex Steud.
Plantago media	Hoary plantain	L.
Polystichum setiferum	Soft shield fern	(Forssk.) T. Moore ex Woyn.
Potamogeton crispus	Curled pond weed	L.
Potamogeton natans	Broad leaved pond weed	L.
Potamogeton polygonifolius	Bog pond weed	Pourr.
Pulicaria dysenterica	Fleabane	(L.)Bernh.
Quercus robur	Pendunculate oak	L.
Ranunculus flammula	Spearwort	L.
Ranunculus repens	Creeping buttercup	L.
Ranunculus aquatilis	Water crowsfoot	L.
Ribes nigrum	Currant	L.
Rhododendron ponticum	Rhododendron	L.
Rorippa amphibia	Yellow cress	(L.)Besser
Rosa canina	Dog rose	L.
Rubus fruticosus	Bramble	L.
Rumex acetosella	Sheep sorrel	L.
Rumex hydrolapathum	Water dock	Huds.
Ruppia cirrhosa	Tasselweed	(Petagna) Grande
Salix caprea	Goat willow	L.
Salix fragilis	Crack willow	L.
Salix repens	Creeping willow	L.
Sambucus nigra	Elder	L.
Schoenoplectus lacustris	Club rush	(L.)Palla
Scutellaria galericulata	Skullcap	L.
Senecio jacobea	Ragwort	L.
Solanum dulcamara	Bittersweet	L.
Sparganium emersum	Branched burr reed	Rehmann
Stachys officinalis	Betony	(L.) Trevis.
Stachys palustris	Marsh Woundwort	L.
Stellaria palustris	Marsh stichweed	Ehrh. ex Hoffm.
Typha angustifolia	Lesser reedmace	L.
Typha latifolia	Greater reed mace	L.
Urtica dioica	Nettle	L.
Veronica beccabunga	Brooklime	L.
Veronica chamaedrys	Germander speedwell	L.
Viola riviniana	Dog violet	Rchb.

Appendix 5 – Plant Species Lists by Sampling Location

Perry 1	Perry 2	Romney 1	Romney 2	Rye Harbour 1	Rye Harbour 2	Rye Harbour 3	Rye Harbour 4	Rye Harbour 5
Glyceria fluitans	*Juncus effusus*	*Hydrocotyle vulgaris*	*Hydrocotyle vulgaris*	*Juncus effusus*	*Juncus effusus*	*Ceratophyllum demersum*	*Eleocharis palustris*	*Crassula helmsii*
Glyceria declinata	*Fagus sylvatica*	*Myosotis scorpioides*	*Crassula helmsii*	*Hydrocotyle vulgaris*	*Crassula helmsii*	*Juncus effusus*	*Hydrocotyle vulgaris*	*Anthemis arvensis*
Iris pseudacorus	*Lemna minor*	*Crassula helmsii*	*Lycopus europaeus*	*Crassula helmsii*	*Eleocharis palustris*	*Hydrocotyle vulgaris*	*Bolboschoenus maritimus*	*Lemna minor*
Rubus fruticosus	*Carex remota*	*Salix caprea*	*Salix caprea*	*Pulicaria dysenterica*	*Rorippa amphibia*	*Crassula helmsii*	*Mentha aquatica*	*Ruppia cirrhosa*
Crassula helmsii	*Nuphar lutea*	*Rubus fruticosus*	*Galium palustre*	*Typha latifolia*	*Glyceria fluitans*	*Schoenoplectus lacustris*	*Crassula helmsii*	*Juncus effusus*
Callitriche stagnalis	*Crassula helmsii*	*Epilobium hirsutum*	*Myosotis scorpioides*	*Eleocharis palustris*	*Cirsium palustre*	*Typha latifolia*	*Myosotis scorpioides*	*Juncus inflexus*
Quercus robur	*Rubus fruticosus*	*Senecio jacobea*	*Argentina anserina*	*Juncus articulatus*	*Hydrocotyle vulgaris*	*Juncus inflexus*	*Juncus effusus*	*Phragmites australis*
Juncus effusus	*Blechnum*	*Anthemis arvensis*	*Rumex hydrolapathum*	*Sparganium emersum*	*Mentha aquatica*	*Eleocharis palustris*	*Typha latifolia*	*Typha latifolia*
Fagus sylvatica	*Rhododendron ponticum*	*Lycopus europaeus*		*Cirsium arvense*	*Lycopus europaeus*	*Argentina anserina*	*Oenanthe fistulosa*	*Glyceria fluitans*
Salix caprea	*Betula pendula*	*Ranunculus repens*		*Lemna minor*	*Cirsium arvense*	*Mentha aquatica*	*Galium palustre*	*Myriophyllum spicatum*
Lemna minor	*Iris pseudacorus*	*Cirsium arvense*			*Lotus corniculatus*	*Persicaria hydropiper*	*Argentina anserina*	
Rhododendron ponticum		*Juncus effusus*			*Pulicaria dysenterica*	*Oenanthe fistulosa*	*Phragmites australis*	
Nuphar lutea		*Juncus articulatus*			*Plantago media*	*Alisma plantago-aquatica*	*Ranunculus flammula*	
Betula pubescens		*Galium palustre*			*Galium palustre*	*Elodea canadensis*	*Alisma plantago-aquatica*	
Carex remota		*Carex riparia*			*Ruppia cirrhosa*	*Lemna trisulca*	*Juncus inflexus*	

Dungeness C1	**Dungeness 1**	**Dungeness 2**	**Dungeness 3**	**Dungeness 4**	**Dungeness 5**	**Oare C1**	**Oare C2**	**Oare 1**
Alisma plantago-aquatica	*Crassula helmsii*	*Lemna trisulca*	*Rumex hydrolapathum*	*Phragmites australis*	*Crassula helmsii*	*Galium palustre*	*Phragmites australis*	*Rumex hydrolapathum*
Lycopus europaeus	*Juncus effusus*	*Crassula helmsii*	*Crassula helmsii*	*Crassula helmsii*	*Lycopus europaeus*	*Epilobium palustre*	*Typha latifolia*	*Typha latifolia*
Rumex hydrolapathum	*Bolboschoenus maritimus*	*Mentha aquatica*	*Mentha aquatica*	*Mentha aquatica*	*Bolboschoenus maritimus*	*Apium nodiflorum*	*Galium palustre*	*Galium palustre*
Juncus effusus	*Pulicaria dysenterica*	*Iris pseudacorus*	*Plantago media*	*Lycopus europaeus*	*Phragmites australis*	*Glyceria fluitans*	*Glyceria fluitans*	*Lemna trisulca*
Iris pseudacorus	*Rumex hydrolapathum*	*Berula erecta*	*Persicaria hydropiper*	*Salix caprea*	*Alisma plantago-aquatica*	*Hippuris vulgaris*	*Persicaria amphibia*	*Apium nodiflorum*
Ceratophyllum demersum	*Iris pseudacorus*	*Ceratophyllum demersum*	*Eleocharis palustris*	*Oenanthe fistulosa*	*Pulicaria dysenterica*	*Phragmites australis*	*Rumex hydrolapathum*	*Solanum dulcamara*
Cirsium arvense	*Typha latifolia*	*Pulicaria dysenterica*	*Carex sp.*	*Juncus effusus*	*Hydrocotyle vulgaris*	*Rumex hydrolapathum*	*Lemna minor*	*Phragmites australis*
Convolvulus arvensis	*Phragmites australis*	*Rumex hydrolapathum*	*Elodea canadensis*	*Alisma plantago-aquatica*	*Mentha aquatica*	*Ranunculus repens*	*Eleocharis palustris*	*Glyceria fluitans*
Persicaria hydropiper	*Lycopus europaeus*	*Juncus effusus*	*Pulicaria dysenterica*	*Rumex hydrolapathum*	*Eleocharis palustris*	*Eleocharis palustris*	*Argentina anserina*	*Hydrocharis morsus-ranae*
Bolboschoenus maritimus	*Myosotis scorpioides*	*Eleocharis palustris*	*Alisma plantago-aquatica*	*Myosotis scorpioides*	*Juncus articulatus*	*Hydrocharis morsus-ranae*		*Juncus effusus*
Elodea canadensis	*Rubus fruticosus*	*Alisma plantago-aquatica*	*Juncus inflexus*	*Bolboschoenus maritimus*	*Carex pendula*	*Juncus inflexus*		*Crassula helmsii*
	Convolvulus arvensis	*Galium palustre*	*Myosotis scorpioides*	*Iris pseudacorus*	*Salix repens*	*Typha latifolia*		*Hippuris vulgaris*
	Ranunculus repens	*Glyceria fluitans*	*Lythrum salicaria*	*Equisetum sp.*	*Epilobium hirsutum*			
	Galium palustre	*Epilobium hirsutum*	*Stachys palustris*	*Cirsium palustre*	*Rumex hydrolapathum*			

Oare 2	**Oare 3**	**Oare 4**	**Oare 5**	**Stodmarch C1**	**Stodmarsh C2**	**Stodmarsh 1**	**Stodmarsh 2**	**Stodmarsh 3**
Solanum dulcamara	*Rumex hydrolapathum*	*Rumex hydrolapathum*	*Bolboschoenus maritimus*	*Phragmites australis*	*Epilobium hirsutum*	*Phragmites australis*	*Juncus inflexus*	*Crassula helmsii*
Glyceria fluitans	*Galium palustre*	*Juncus inflexus*	*Rumex hydrolapathum*	*Lemna minor*	*Phragmites australis*	*Eleocharis palustris*	*Alisma plantago-aquatica*	*Phragmites australis*
Bolboschoenus maritimus	*Hydrocharis morsus-ranae*	*Glyceria fluitans*	*Hippuris vulgaris*	*Rumex hydrolapathum*	*Hydrocharis morsus-ranae*	*Crassula helmsii*	*Crassula helmsii*	*Epilobium palustre*
Phragmites australis	*Apium nodiflorum*	*Bolboschoenus maritimus*	*Eleocharis palustris*	*Lemna trisulca*	*Potamogeton natans*	*Myriophyllum spicatum*	*Phragmites australis*	*Typha latifolia*
Rumex hydrolapathum	*Bolboschoenus maritimus*	*Eleocharis palustris*	*Lemna trisulca*	*Cirsium arvense*	*Rumex hydrolapathum*	*Epilobium palustre*	*Glyceria fluitans*	*Ranunculus aquatilis*
Hydrocharis morsus-ranae	*Eleocharis palustris*	*Typha latifolia*	*Lemna minor*	*Epilobium hirsutum*	*Glyceria fluitans*	*Dipsacus fullonum*	*Eleocharis palustris*	*Alisma plantago-aquatica*
Lemna trisulca	*Juncus inflexus*	*Apium nodiflorum*	*Glyceria fluitans*	*Carex nigra*	*Cirsium arvense*	*Glyceria fluitans*	*Dipsacus fullonum*	*Ranunculus repens*
Apium nodiflorum	*Crassula helmsii*	*Hydrocharis morsus-ranae*	*Hydrocharis morsus-ranae*	*Typha latifolia*	*Dipsacus fullonum*	*Ranunculus repens*	*Myriophyllum spicatum*	*Juncus inflexus*
Typha latifolia	*Typha latifolia*	*Crassula helmsii*	*Typha latifolia*	*Pulicaria dysenterica*	*Persicaria amphibia*	*Rorippa amphibia*		*Glyceria fluitans*
Crassula helmsii	*Lemna trisulca*	*Lemna trisulca*	*Juncus inflexus*	*Glyceria fluitans*	*Convolvulus arvensis*	*Juncus inflexus*		*Lemna trisulca*
Galium palustre	*Solanum dulcamara*		*Crassula helmsii*		*Urtica dioica*	*Pulicaria dysenterica*		*Hippuris vulgaris*
Epilobium palustre			*Apium nodiflorum*		*Ranunculus repens*	*Typha latifolia*		*Rumex hydrolapathum*
Eleocharis palustris					*Juncus inflexus*			*Elodea canadensis*
								Mentha aquatica
								Eleocharis palustris
								Cirsium arvense

Stodmarsh 4	**Stodmarsh 5**	**Eastry 1**	**Eastry 2**	**Fowlmead C1**	**Fowlmead C2**	**Fowlmead C3**	**Blean 1**	**Blean 2**
Alisma plantago-aquatica	*Phragmites australis*	*Typha latifolia*	*Glyceria fluitans*	*Carex nigra*	*Glyceria fluitans*	*Typha latifolia*	*Quercus robur*	*Quercus robur*
Hippuris vulgaris	*Crassula helmsii*	*Caltha palustris*	*Carex nigra*	*Mentha aquatica*	*Typha latifolia*	*Ranunculus flammula*	*Alnus*	*Fraxinus excelsior*
Rumex hydrolapathum	*Glyceria fluitans*	*Iris pseudacorus*	*Rosa canina*	*Phragmites australis*	*Juncus inflexus*	*Rumex hydrolapathum*	*Salix caprea*	*Ajuga reptans*
Phragmites australis	*Eleocharis palustris*	*Lemna minor*	*Carex demissa*	*Rubus fruticosus*	*Elodea canadensis*	*Elodea canadensis*	*Juncus effusus*	*Juncus effusus*
Iris pseudacorus	*Rumex hydrolapathum*	*Salix caprea*	*Alisma Plantago-aquatica*	*Epilobium palustre*	*Juncus effusus*	*Potamogeton crispus*	*Eleocharis palustris*	*Crassula helmsii*
Crassula helmsii	*Typha latifolia*	*Alisma plantago-aquatica*	*Argentina anserina*	*Solanum dulcamara*	*Rumex hydrolapathum*	*Juncus effusus*	*Rubus fruticosus*	*Lemna minor*
Ranunculus repens	*Alisma plantago-aquatica*	*Glyceria fluitans*	*Rumex hydrolapathum*	*Typha latifolia*	*Myosotis scorpioides*	*Salix caprea*	*Ranunculus repens*	*Potamogeton natans*
Typha latifolia	*Potamogeton natans*	*Crassula helmsii*	*Epilobium hirsutum*	*Juncus inflexus*	*Pulicaria dysenterica*	*Pulicaria dysenterica*	*Crassula helmsii*	*Salix caprea*
Lotus corniculatus	*Hydrocharis morsus-ranae*	*Epilobium palustre*	*Convolvulus arvensis*	*Glyceria fluitans*	*Potamogeton natans*	*Juncus inflexus*	*Potamogeton natans*	*Rumex hydrolapathum*
Eleocharis palustris	*Iris pseudacorus*	*Epilobium hirsutum*	*Carex pendulous*	*Argentina anserina*	*Salix fragilis*	*Eleocharis palustris*	*Lemna minor*	*Galium palustre*
Epilobium palustre	*Sparganium natans*	*Urtica dioica*	*Menyanthes trifoliata*	*Holcus lanatus*	*Epilobium palustre*	*Glyceria fluitans*	*Viola riviniana*	*Callitriche stagnalis*
Anthemis arvensis	*Hippuris vulgaris*	*Convolvulus arvensis*	*Myriophyllum spicatum*		*Eleocharis palustris*	*Caltha palustris*	*Hedera helix*	*Glyceria fluitans*
Persicaria hydropiper	*Pulicaria dysenterica*	*Myriophyllum spicatum*	*Salix caprea*		*Salix caprea*	*Carex pendulous*	*Galium palustre*	*Viola riviniana*
	Mentha aquatica	*Rumex hydrolapathum*	*Juncus effusus*		*Lotus corniculatus*	*Ranunculus repens*	*Lotus corniculatus*	*Hedera helix*
	Myosotis scorpioides	*Ribes nigrum*	*Rubus fruticosus*		*Alisma Plantago-aquatica*	*Iris pseudacorus*	*Glyceria fluitans*	*Rubus fruticosus*
	Lotus corniculatus	*Carex pendulous*	*Epilobium palustre*		*Ranunculus repens*	*Salix fragilis*		
	Lemna trisulca	*Gallium aparine*	*Caltha palustris*		*Carex pendulous*	*Potamogeton natans*		
	Juncus inflexus	*Plantago media*	*Urtica dioica*					
	Carex nigra	*Solanum dulcamara*	*Mentha aquatica*					
		Eleocharis palustris	*Gallium aparine*					
		Carex demissa	*Ranunculus repens*					
			Crassula helmsii					

Hothfield C1	**Hothfield C2**	**Hothfield C3**	**Hothfield C4**	**Hothfield 1**	**Hothfield 2**	**Sevenoaks C1**	**Sevenoaks 1**	**Sevenoaks 2**
Ranunculus flammula	*Mentha aquatica*	*Mentha aquatica*	*Rumex hydrolapathum*	*Rumex hydrolapathum*	*Typha latifolia*	*Typha latifolia*	*Alnus glutinosa*	*Crassula helmsii*
Callitriche stagnalis	*Ranunculus repens*	*Juncus effusus*	*Juncus effusus*	*Mentha aquatica*	*Carex paniculata*	*Alnus glutinosa*	*Crassula helmsii*	*Mentha aquatica*
Juncus effusus	*Ranunculus flammula*	*Myosotis scorpioides*	*Ranunculus repens*	*Menyanthes trifoliata*	*Hydrocotyle vulgaris*	*Elodea canadensis*	*Epilobium hirsutum*	*Epilobium hirsutum*
Ranunculus repens	*Rorippa amphibia*	*Lychnis flos-cuculi*	*Berula erecta*	*Salix caprea*	*Hypericum elodes*	*Lysimachia nummularia*	*Epilobium palustre*	*Epilobium palustre*
Galium palustre	*Epilobium palustre*	*Salix caprea*	*Ranunculus flammula*	*Juncus effusus*	*Cardamine pratensis*	*Nuphar lutea*	*Typha latifolia*	*Hypericum elodes*
Alisma plantago-aquatica	*Galium palustre*	*Betula pubescens*	*Myosotis scorpioides*	*Typha latifolia*	*Juncus inflexus*	*Convolvulus arvensis*	*Juncus inflexus*	*Pulicaria dysenterica*
Fraxinus excelsior	*Urtica dioica*	*Rumex acetosella*	*Hydrocotyle vulgaris*	*Ranunculus repens*	*Crassula helmsii*	*Myosotis scorpioides*	*Mentha aquatica*	*Alnus glutinosa*
Epilobium hirsutum	*Acer pseudoplatanus*	*Epilobium hirsutum*	*Callitriche stagnalis*	*Galium palustre*	*Juncus effusus*	*Lycopus europaeus*	*Lotus corniculatus*	*Lysimachia nummularia*
Veronica chamaedrys	*Rumex hydrolapathum*	*Hypericum elodes*	*Salix caprea*	*Rubus fruticosus*	*Ranunculus repens*	*Pulicaria dysenterica*	*Argentina anserina*	*Myosotis scorpioides*
Typha latifolia	*Callitriche stagnalis*	*Rumex hydrolapathum*	*Typha latifolia*	*Rumex acetosella*	*Salix caprea*	*Epilobium hirsutum*	*Lysimachia nummularia*	*Rorippa amphibia*
Iris pseudacorus	*Juncus effusus*	*Lotus corniculatus*	*Mentha aquatica*	*Holcus lanatus*	*Galium palustre*	*Carex pendulous*	*Phragmites australis*	*Ajuga reptans*
Rorippa amphibia	*Typha latifolia*	*Cirsium palustre*	*Epilobium palustre*	*Crassula helmsii*	*Rumex hydrolapathum*	*Argentina anserina*	*Lythrum salicaria*	*Persicaria hydropiper*
Caltha palustris	*Iris pseudacorus*	*Ranunculus flammula*	*Carex paniculata*	*Myosotis scorpioides*	*Mentha aquatica*	*Lemna trisulca*	*Urtica dioica*	*Scutellaria galericulata*
Berula erecta	*Phalaris arundinacea*	*Ranunculus repens*	*Galium palustre*	*Juncus articulatus*	*Juncus articulatus*		*Myosotis scorpioides*	*Lycopus europaeus*
Rumex hydrolapathum	*Betula pendula*	*Berula erecta*	*Cirsium palustre*	*Carex paniculata*	*Lotus corniculatus*		*Rumex hydrolapathum*	*Lotus corniculatus*
Potamogeton natans	*Ribes nigrum*	*Epilobium palustre*	*Rubus fruticosus*	*Epilobium hirsutum*	*Epilobium hirsutum*		*Stachys officinalis*	*Centaurium pulchellum*
		Carex paniculata		*Lemna minor*	*Menyanthes trifoliata*		*Pulicaria dysenterica*	
		Typha latifolia		*Hydrocotyle vulgaris*	*Berula erecta*			
		Galium palustre		*Lotus corniculatus*				
		Stellaria palustris						
		Juncus articulatus						
		Carex echinata						

Sevenoaks 3	**Sevenoaks 4**	**Sevenoaks 5**	**Bough Beech C1**	**Bough Beech 1**	**Bough Beech 2**	**Bough Beech 3**	**Bough Beech 4**	**Bough Beech 5**
Crassula helmsii	*Ceratophyllum demersum*	*Crassula helmsii*	*Lemna minor*	*Juncus effusus*	*Crassula helmsii*	*Crassula helmsii*	*Convolvulus arvensis*	*Crassula helmsii*
Epilobium hirsutum	*Crassula helmsii*	*Typha latifolia*	*Lemna trisulca*	*Myosotis scorpioides*	*Mentha aquatica*	*Juncus effusus*	*Crassula helmsii*	*Argentina anserina*
Mentha aquatica	*Mentha aquatica*	*Lemna trisulca*	*Ranunculus flammula*	*Epilobium hirsutum*	*Juncus effusus*	*Lemna minor*	*Myosotis scorpioides*	*Lysimachia nummularia*
Rumex hydrolapathum	*Menyanthes trifoliata*	*Alnus glutinosa*	*Iris pseudacorus*	*Epilobium palustre*	*Epilobium hirsutum*	*Salix caprea*	*Urtica dioica*	*Alisma plantago-aquatica*
Lycopus europaeus	*Typha latifolia*	*Menyanthes trifoliata*	*Berula erecta*	*Crassula helmsii*	*Lycopus europaeus*	*Alnus glutinosa*	*Lemna minor*	*Lemna minor*
Alnus glutinosa	*Lemna minor*	*Lycopus europaeus*	*Bolboschoenus maritimus*	*Argentina anserina*	*Myosotis scorpioides*	*Persicaria hydropiper*	*Alisma plantago-aquatica*	*Salix caprea*
Urtica dioica	*Epilobium hirsutum*	*Lythrum salicaria*	*Alisma Plantago-aquatica*	*Mentha aquatica*	*Argentina anserina*	*Mentha aquatica*	*Argentina anserina*	*Convolvulus arvensis*
Lythrum salicaria	*Alisma plantago-aquatica*	*Juncus inflexus*	*Juncus effusus*	*Lycopus europaeus*	*Juncus inflexus*	*Epilobium hirsutum*	*Iris pseudacorus*	*Iris pseudacorus*
Convolvulus arvensis	*Urtica dioica*	*Mentha aquatica*	*Solanum dulcamara*	*Persicaria hydropiper*	*Salix caprea*	*Convolvulus arvensis*	*Mentha aquatica*	*Rubus fruticosus*
Elodea canadensis	*Ranunculus repens*	*Lemna minor*	*Lotus corniculatus*	*Alisma plantago-aquatica*	*Lysimachia nummularia*	*Carex nigra*	*Salix caprea*	*Epilobium hirsutum*
Ruppia cirrhosa	*Lythrum salicaria*	*Elodea canadensis*	*Lycopus europaeus*	*Juncus articulatus*	*Glyceria fluitans*	*Lysimachia nummularia*	*Carex pendulous*	*Myosotis scorpioides*
Persicaria hydropiper	*Alnus glutinosa*	*Juncus effusus*	*Pulicaria dysenterica*			*Argentina anserina*	*Lycopus europaeus*	
	Salix fragilis	*Salix fragilis*	*Cirsium arvense*			*Lycopus europaeus*	*Epilobium hirsutum*	
	Carex pendulous	*Ceratophyllum demersum*	*Typha latifolia*			*Juncus inflexus*	*Rumex hydrolapathum*	
	Galium palustre	*Epilobium hirsutum*	*Mentha aquatica*					
	Betula pendula		*Rumex hydrolapathum*					
			Argentina anserina					

Oare GWC1	**Oare GWC2**	**Oare GWC3**	**Beacon 1**	**Beacon 2**	**Bewl 1**	**Bewl 2**	**Bewl 3**	**Bewl 4**
Lemna minor	*Circaea lutetiana*	*Aesculus hippocastanum*	*Eleocharis palustris*	*Crassula helmsii*	*Juncus effusus*	*Crassula helmsii*	*Crassula helmsii*	*Crassula helmsii*
Hydrocharis morsus-ranae	*Lemna minor*	*Rumex hydrolapathum*	*Juncus articulatus*	*Phragmites australis*	*Berula erecta*	*Salix caprea*	*Salix caprea*	*Argentina anserina*
Ceratophyllum demersum	*Urtica dioica*	*Blechnum spicant*	*Alnus glutinosa*	*Eleocharis palustris*	*Salix caprea*	*Rorippa amphibia*	*Argentina anserina*	*Salix caprea*
Urtica dioica	*Hedera helix*	*Rubus fruticosus*	*Phragmites australis*	*Salix caprea*	*Mentha aquatica*	*Mentha aquatica*	*Persicaria hydropiper*	*Eleocharis palustris*
Salix caprea	*Berula erecta*	*Lemna minor*	*Glyceria fluitans*	*Glyceria fluitans*	*Apium nodiflorum*	*Berula erecta*	*Rorippa amphibia*	*Persicaria hydropiper*
Epilobium hirsutum	*Iris pseudacorus*	*Urtica dioica*	*Alisma Plantago-aquatica*	*Callitriche stagnalis*	*Crassula helmsii*	*Glyceria fluitans*	*Lycopus europaeus*	
Phragmites australis	*Rubus fruticosus*	*Carex pendulous*	*Crassula helmsii*	*Bolboschoenus maritimus*	*Epilobium hirsutum*	*Typha latifolia*	*Oenanthe fistulosa*	
Geranium	*Acer pseudoplatanus*	*Circaea lutetiana*			*Eleocharis palustris*	*Eleocharis palustris*		
Solanum dulcamara		*Fraxinus excelsior*			*Rumex hydrolapathum*	*Rumex hydrolapathum*		
Aesculus hippocastanum		*Sambucus nigra*			*Persicaria hydropiper*	*Argentina anserina*		
		Acer pseudoplatanus			*Argentina anserina*	*Juncus effusus*		
		Hedera helix			*Rorippa amphibia*			
		Veronica beccabunga			*Glyceria fluitans*			
		Polystichum setiferum						

Bewl 5	**Rye Street C1**	**Rye Street C2**	**Rye Street 1**	**Rye Street 2**	**Rye Street 3**	**Rye Street 4**	**Rye Street 5**	**Shorne 1**
Persicaria hydropiper	*Lemna trisulca*	*Eleocharis palustris*	*Bolboschoenus maritimus*	*Bolboschoenus maritimus*	*Eleocharis palustris*	*Typha latifolia*	*Epilobium palustre*	*Crassula helmsii*
Argentina anserina	*Ranunculus aquatilis*	*Lemna trisulca*	*Eleocharis palustris*	*Lemna trisulca*	*Bolboschoenus maritimus*	*Crassula helmsii*	*Oenanthe fistulosa*	*Alnus glutinosa*
Salix caprea	*Bolboschoenus maritimus*	*Bolboschoenus maritimus*	*Lemna trisulca*	*Lemna minor*	*Crassula helmsii*	*Lycopus europaeus*	*Crassula helmsii*	*Carex pendulous*
Rorippa amphibia	*Rumex hydrolapathum*	*Rumex hydrolapathum*	*Crassula helmsii*	*Glyceria fluitans*	*Lemna minor*	*Oenanthe fistulosa*	*Bolboschoenus maritimus*	*Phragmites australis*
Berula erecta	*Crataegus monogyna*	*Glyceria fluitans*	*Lemna minor*	*Crassula helmsii*	*Lemna trisulca*	*Berula erecta*	*Berula erecta*	*Salix caprea*
Eleocharis palustris			*Argentina anserina*	*Ranunculus aquatilis*	*Phragmites australis*	*Rumex hydrolapathum*	*Rumex hydrolapathum*	*Lemna minor*
Glyceria fluitans			*Lycopus europaeus*	*Rumex hydrolapathum*	*Rumex hydrolapathum*	*Solanum dulcamara*	*Ranunculus aquatilis*	
			Oenanthe fistulosa		*Glyceria fluitans*	*Eleocharis palustris*	*Glyceria fluitans*	
					Apium nodiflorum	*Phalaris arundinacea*	*Eleocharis palustris*	
					Ranunculus aquatilis		*Solanum dulcamara*	
					Myosotis scorpioides			

Shorne 2	**Shorne 3**	**Shorne 4**	**Shorne 5**	**Orlestone 1**	**Orlestone 2**
Juncus effusus	*Phragmites australis*	*Glyceria declinata*	*Crassula helmsii*	*Betula pendula*	*Potamogeton polygonifolius*
Crassula helmsii	*Juncus articulatus*	*Juncus effusus*	*Alnus glutinosa*	*Salix caprea*	*Sparganium emersum*
Rubus fruticosus	*Crassula helmsii*	*Eleocharis palustris*	*Bolboschoenus maritimus*	*Typha angustifolia*	*Salix caprea*
Betula pubescens	*Epilobium palustre*	*Crassula helmsii*	*Typha latifolia*	*Typha latifolia*	*Juncus effusus*
Mentha aquatica	*Salix caprea*	*Glyceria fluitans*	*Myriophyllum spicatum*	*Sparganium emersum*	*Lemna minor*
Phragmites australis	*Lotus corniculatus*	*Salix caprea*	*Potamogeton natans*	*Juncus effusus*	*Typha angustifolia*
Phalaris arundinacea	*Alisma Plantago-aquatica*		*Potamogeton crispus*	*Juncus articulatus*	*Typha latifolia*
Lemna minor	*Typha latifolia*		*Nuphar lutea*	*Rubus fruticosus*	*Betula pendula*
Lemna trisulca	*Senecio jacobea*			*Glyceria fluitans*	
	Juncus effusus				